UNIVERSITÉ DE LA ROCHELLE

Ecole Doctorale Sciences pour l'Environnement, Gay Lussac

Structures sociale, écologique et génétique du grand dauphin, *Tursiops truncatus*, dans le golfe Normand-Breton et dans l'Atlantique Nord-Est

Social, ecological and genetic structures of bottlenose dolphins, Tursiops truncatus, in the Normano-Breton gulf and in the North-East Atlantic

Thèse présentée par Marie LOUIS

Soutenue le 15 Juillet 2014

pour l'obtention du grade de Docteur de l'Université de La Rochelle

Discipline : Biologie de l'environnement, des populations, écologie marine

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Glossary

The terms defined in the glossary will be highlighted by an asterisk (*) when first used in the text.

- Allele richness: is a measure of the number of alleles that takes into account variations in sample size.

- Bottleneck: drastic reduction of the effective population size* of a population.

- Complete lineage sorting: is the segregation of alleles/haplotypes among populations.

- Effective population size (N_e): is the number of breeders in an idealized population (in Hardy-Weinberg equilibrium*) that would show the same amount of genetic drift* and inbreeding than the population under consideration.

- Founder event: also called founder effect, occurs when a small group of individuals become isolated from the rest of the population.

- Genetic drift: random variation of allele frequencies.

- Haplotypic diversity: it measures the probability that two randomly chosen sequences in a population will be the same.

- Hardy-Weinberg Equilibrium (HWE): this principle states that the genetic variation of a population will remain constant from one generation to the next in the absence of selection, mutation, migration and genetic drift*. It assumes that there are panmixia and non-overlapping generations.

- Linkage equilibrium: random associations of alleles.

- Nucleotide diversity: it measures the average proportion of nucleotide differences between all pairs of sequences within a population.

- Null allele: a null allele (at a microsatellite locus) is an allele which is present in a sample but which consistently fails to amplify during polymerase chain reaction (PCR). Amplification of the allele can be inhibited because of a mutation in the primer binding region.

- Private alleles: alleles that are only found in one population.

- Wahlund effect: it is a reduction in the overall heterozygosity as a result of population structure.

CHAPTER

1

GENERAL INTRODUCTION

1) Interaction between social, ecological and genetic structures

Biodiversity is not spread homogeneously on earth. Its distribution is driven by physical factors such as climatic or environmental conditions and biological factors such as the presence of competitors, conspecifics or prey (Lomolino *et al.* 2006). As a result of this non-random distribution, individuals and their conspecifics do not have the same chance of encounter, to interact or reproduce with each other. At a fine-scale level, individuals do not interact randomly with each other. Groups of individuals may tend to form aggregations due to the availability of food, shelter, and resting areas. Additionally, they can also form social groups where individuals actively seek or maintain proximity with each other or will receive benefits from living with others (Whitehead 2008a). Social structure describes the patterns of these interactions (or associations) between individuals (Hinde 1976) and details the number and characteristics of the individuals in a group as well as the duration and the nature of their interactions. At fine to large scales, as resources (i.e. habitat and diet) are not evenly distributed, they may be used differently by individuals, resulting in ecological structure. In addition, individuals do not always mate randomly with each other, which creates genetic structure. They can form populations that are sets of individuals that preferentially breed among themselves than with other individuals ("the evolutionary definition of a population", Waples & Gaggiotti 2006).

In non-social animal species, ecological and genetic structures may influence each other. In social species, such as large mammals, social, ecological and genetic structures are strongly interlinked and may interact with each other. First, social structure can match ecological structure if in a social group, foraging techniques are transmitted socially. This was recorded across different species, including sperm whales, killer whales or pilot whales, where individuals within the same social group demonstrated more similar ecology than individuals from different groups (Marcoux *et al.* 2007; de Stephanis *et al.* 2008b; Riesch *et al.* 2012). This may reflect differences in cultural traditions of habitat use and food choice. Likewise, bottlenose dolphins in Moreton Bay, which have different hunting strategies (interacting or not with trawl fisheries) have formed two distinct social clusters (Chilvers &

Corkeron 2001). In turn, if individuals preferentially interact with individuals having similar ecology, ecological behavior might shape social structure. For instance, when trawl fisheries were banned in Moreton Bay, trawler and non-trawler bottlenose dolphins no longer formed two separate social clusters, highlighting the possible effect of the disappearance of ecological differences on social structure (Ansmann *et al.* 2012a; Cantor & Whitehead 2013).

Social structure can also influence patterns of gene flow (see review in Sugg *et al.* 1996; Storz 1999). In particular, low dispersal or sex-biased dispersal, mating systems such as polygyny can lead to genetic differentiation among social groups (Storz 1999). Populations can be composed by sub-groups of varying degrees of relatedness or co-ancestry, which are important to take into account in population genetics whose models are based on random mating (Sugg *et al.* 1996). For instance, red howler monkeys had a polygynous mating system and moderate female philopatry which created genetic differentiation among adjacent groups (Pope 1992). Female matrilocality or philopatry can lead to genetic differentiation among groups while mating can still be random when males disperse (e.g. for sheep, Coltman *et al.* 2003). Similarly, in matrilineal pilot whale or killer whale groups, although both males and females stay in their natal groups, males do not generally mate with females inside their group which leads to gene flow among groups (Amos *et al.* 1993; Pilot *et al.* 2010). Nevertheless, genetic structure among populations or ecotypes of killer whales may be strengthened by the kin structure of social groups (Pilot *et al.* 2010).

Social structure most likely influenced genetic structure along with others factors such as geographic or ecological barriers to gene flow. For instance, a combination of distinct social structure and roosting ecology may lead to different patterns of genetic structure among seven bats species inhabiting an undisturbed ancient rainforest, therefore controlling for historical processes (Rossiter *et al.* 2012). Ecological structure, in terms of variation of habitats or diet may lead to genetic structure. For instance, patterns of genetic divergence in highly mobile carnivores such as wolves and coyotes were correlated with differences in habitats and/or diet (Sacks *et al.* 2004; Sacks *et al.* 2005; Pilot *et al.* 2006; Musiani *et al.* 2007; Sacks *et al.* 2008; Pilot *et al.* 2012). Individuals may have a higher tendency to disperse in familiar habitats (i.e. natal habitat dispersal, Davis & Stamps 2004) where they may be able to use foraging techniques learned during juvenile life or target familiar prey, which will likely increase their foraging success and thus their fitness. Social structure and long-term mother- calf bonds may strengthen this pattern (Sacks *et al.* 2005; Musiani *et al.* 2007; Pilot *et*

al. 2010). For instance, killer whale genetic differentiation between offshore, transient and resident ecotypes may be maintained by learned foraging techniques in the matrilineal group and tight social bonds (Hoelzel *et al.* 1998a; Hoelzel *et al.* 2007; Pilot *et al.* 2010; Riesch *et al.* 2012). More generally, long-term niche specializations[1], in terms or habitats or diet, among groups of individuals, can facilitate the evolution and maintenance of genetic divergence both for non-social and social species (Smith & Skúlason 1996; Bolnick *et al.* 2003; Knudsen *et al.* 2010; Siwertsson *et al.* 2013).

Social, ecological and genetic structures are therefore tightly inter-connected. Although often rarely studied together, combining these approaches is essential for a global understanding of the structuring patterns of social species. Different processes are creating and maintaining these different levels of structure in the animal kingdom. This introduction chapter gives an overview of the mechanisms involved. The importance of studying the structure of populations for conservation purposes is highlighted. The context of the study and its objectives are presented and the organization of the manuscript is outlined.

2) Drivers of structure

Interactions between intrinsic behavioral factors and extrinsic environmental factors shape the different levels of structure. I will discuss possible mechanisms that influence sociality, ecological structure and barriers to gene flow.

a) Social structure

On a fine-scale, individuals usually associate non-randomly with other individuals. They may preferentially associate with others that share similar traits, a phenomenon which is called homophily or assortativity. These associations could be according to age (e.g. Blumstein 2012; Hauver *et al.* 2013), sex (reviewed by Ruckstuhl 2007), morphological traits

[1] We define a niche (or an ecological) specialization as the act of exploiting only a limited fraction of the range of available feeding or habitat resources (Bolnick *et al.* 2003).

(e.g. body length, Croft *et al.* 2005; Mourier *et al.* 2012), behavior (e.g. Mann *et al.* 2012), kinship (e.g. Holekamp *et al.* 1997; Archie *et al.* 2006), reproductive state (e.g. Sundaresan *et al.* 2007; Möller & Harcourt 2008), previous familiarity (Garroway *et al.* 2013) or personality (e.g. Weinstein & Capitanio 2008; Croft *et al.* 2009; Aplin *et al.* 2013). These relationships among individuals likely reveal behavioral strategies that should maximize fitness (van Schaik 1989). Possible advantages of living in groups include decreased predation risks, cooperation to catch and defend resources, transfer of information and care for another. On the other hand, disadvantages include competition for resources, being more conspicuous and increased aggression rates (see detailed review in Krause & Ruxton 2002). Individuals tend to form groups when benefits outweigh the costs. This trade-off is strongly influenced by predation risks and the availability of resources including both food and access to mates (Alexander 1974; Rubenstein & Wrangham 1986). For instance, individuals tend to form larger groups when predation risks are high (e.g. Wrona & Dixon 1991; Hill & Lee 1998) or when food is abundant (e.g. Chapman *et al.* 1995; Lusseau *et al.* 2004; Smith *et al.* 2008). However, additional factors such as protection of young and defense of territories may explain group sizes (e.g. for females lions, Packer *et al.* 1990). The fitness costs and benefits can also vary according to gender because of different potential rates of reproduction between males and females. In mammals, female sociality is influenced by food resources and protection of young while males compete or cooperate for access to females (Trivers 1972; Emlen & Oring 1977; Wrangham 1980; Clutton-Brock & Parker 1992).

Social dynamics can evolve in response to ecological factors. Variations in food availability can modify association patterns within a population. For instance, female bonobos showed cyclical changes in their association patterns according to the availability of resources. When food was abundant, association strength was lower than when food was scarce (Henzi *et al.* 2009). Social cohesion of resident killer whales and African elephants were found to be higher in seasons with high food abundance (Wittemyer *et al.* 2005, Foster *et al.* 2012). Social structure can also be variable for a given species according to the environment. Guppies from high predation areas had stronger and longer social ties than those from populations from areas where the predation risk was low (Kelley *et al.* 2011). Spinner dolphins in the Main Hawaii islands have a typical fission-fusion social structure (where although some associations can be long-lasting, association patterns are mainly dynamic and

have an hourly or daily turn-over) while individuals in a remote Hawaiian atoll formed a society with stable bonds. This stable social structure may be explained by the isolation of this atoll, and the low availability of resting places, separated by large open areas of pelagic waters with high predation risks (Karczmarski *et al.* 2005). The number of resting sites (i.e. roosting leaves) seemed also to influence leaf roosting bat social structures (Chaverri 2010).

b) Ecological structure

Ecological structure can arise because of environmental characteristics and behavioral processes. Spatially segregated individuals may face different environmental conditions and thus have distinct foraging behavior or diet (e.g. marine and freshwater otters, Kruuk 1995). In addition, resources between individuals may be partitioned to limit intra-specific competition and may be shaped by frequency dependent selection (e.g. Roughgarden 1972; Skúlason & Smith 1995; Bolnick 2001). Already existing diversities in habitats and resources or newly available habitats, created by changes in environmental conditions or the colonization of new territories, might lead to niche specializations (e.g. Smith & Skúlason 1996; Hewitt 2000; Losos & Ricklefs 2009). Habitat release during postglacial periods has opened up ecological opportunities. For instance, ecotype differentiation between benthic and limnetic sticklebacks in post-glacial lakes likely resulted from double invasion events which may be linked to two separate marine submergence events (Taylor & McPhail 2000). Key innovations and extinction of antagonists might also enable individuals to exploit new resources (reviewed in Yoder *et al.* 2010). Individual behavior can also lead to intra-specific ecological variation. For instance, site fidelity to particular feeding or breeding grounds, which may be imprinted (e.g. bluefin tuna) or transmitted through calf's early maternal experience (e.g. baleen whales) can create ecological structure (Rooker *et al.* 2008a; Rooker *et al.* 2008b; Valenzuela *et al.* 2009; Witteveen *et al.* 2009). In social species, ecological specializations can be maintained by vertical learning during juvenile life (e.g. Krützen *et al.* 2005; Sargeant & Mann 2009). Finally, niche specializations may arise as a result of individual plasticity in both behavioral and morphological traits and could be maintained by individual stability in feeding behavior (Bolnick *et al.* 2003; Knudsen *et al.* 2010). In turn, variations in morphological traits associated with feeding can also be the results of adaptations to distinct resources (Smith & Skúlason 1996).

c) Genetic structure

Barriers to gene flow may arise as a result of a complex interaction between environmental, historical and behavioral processes. First, they can evolve in allopatry (Mayr 1942) when groups of individuals are isolated in discontinuous regions separated by mountains, water masses, habitats of poor quality or human constructions, restricting dispersal (e.g. Piertney *et al.* 1998; Gerlach & Musolf 2000; Funk *et al.* 2005). However, genetic structure may also arise when there is no obvious geographic barrier to gene flow. Distance can create genetic differentiation as the majority of individuals usually disperse in a range that is smaller than the whole species range (Slatkin 1993). In addition, past changes in environmental conditions such as Pleistocene climatic oscillations have shaped the genetic structure and diversity of many taxa. In the Northern Hemisphere, temperate species were isolated in refugia during glacial periods and expanded during interglacial periods, which affected genetic diversity patterns (see review in Hewitt 1996, 2000). Moreover, environmental variations such as cryptic or complex habitat breaks, or environmental characteristics such as climate or particular oceanographic features (e.g. currents, salinity and temperature) can reduce dispersal and may explain genetic structure at large and fine scales (Rueness *et al.* 2003; Guillot *et al.* 2005; Jorgensen *et al.* 2005; Coulon *et al.* 2006; Galindo *et al.* 2006; Geffen *et al.* 2007; Gaggiotti *et al.* 2009; Selkoe *et al.* 2010). It has been demonstrated theoretically that environmental gradients may facilitate genetic divergence (Doebeli & Dieckmann 2003). As detailed in the first part of the introduction, a combination of ecology, in particular foraging specializations, and social behavior, leading to philopatry or natal-biased dispersal, may explain genetic divergence of highly mobile vertebrates (e.g. coyotes, wolves or killer whales, Sacks *et al.* 2005; Hoelzel *et al.* 2007; Musiani *et al.* 2007). Natal-biased dispersal patterns hold for both males and females in some species, but in most mammal species dispersal is male biased. Female fitness is constrained by foraging resources, while males tend to maximize their access to females (reviewed in Greenwood 1980; Handley & Perrin 2007).

Genetic isolation between populations with limited gene flow can be enhanced by genetic drift* or by selective pressures. Natural selection can facilitate the evolution of traits adapted to particular environments which will confer higher fitness to individuals in their local habitats (i.e. a phenomenon called “local adaptation”, Kawecki & Ebert 2004).

Ultimately, ecological speciation occurs when reproductive isolation evolves as a consequence of these local adaptations (Schluter 2001; Rundle & Nosil 2005).

Studying these different levels of structure can reveal how ecology and evolution shaped the current patterns of biodiversity and is therefore of major interest in fundamental and theoretical perspectives. In addition, these studies can also have very practical implications for conservation.

3) Conservation implications

A fundamental question in conservation biology is how to delineate conservation units to maintain the adaptive potential of a species and its persistence. It is well accepted that the conservation of many distinct populations will contribute to maximizing evolutionary potential while minimizing the risk of extinction. In addition, a comprehensive understanding of the structure of populations is particularly important for conservation. However, there is a lack of current consensus on which type of structure and time scales are relevant to management.

Genetic structure had a major role in conservation plans. Ecological structure has also been included, but to a lesser extent. Two units have mainly been considered: Evolutionary Significant Units (ESU) and Managements Units (MU). ESU have been defined several times in two different ways involving only genetics for the first definition (neutral diversity) and both genetic and ecology (adaptive variation) for the second. Moritz (1994, 2002) defined them, using only genetics, as units arising from “historical population structure rather than current adaptation that are reciprocally monophyletic for mitochondrial DNA and show significant divergence of allele frequencies at nuclear loci”. In contrast, ESU, according to Crandall *et al.* (2000) are defined when both “ecological and genetic exchangeability” are rejected (i.e. when there is respectively population differentiation caused by genetic drift and

selection and evidence of limited gene flow between populations). They argued that both ecological data and genetic variation of adaptive significance should be used to define ESU.

Management units have been defined by Moritz (1994) as "population units with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles". This definition has often been interpreted as rejecting panmixia between the units, which was criticized as not being reliable or sufficient (Taylor & Dizon 1999; Palsbøll *et al.* 2007). Palsbøll *et al.* (2007) emphasized that management units should correspond to demographically independent populations whose population dynamics are driven by local birth and mortality rates rather than just rejecting panmixia. Current dispersal is the parameter of interest. An analytical framework that will integrate and estimate both population genetics and demographic parameters is needed (Palsbøll *et al.* 2007). However, there is also no consensus on the level at which populations become demographically correlated (Waples & Gaggiotti 2006; Palsbøll *et al.* 2007).

Another issue related to the genetic delineation of management units is that the absence of population genetic structure at neutral loci (e.g. microsatellites or mitochondrial DNA) does not mean that there is no adaptive divergence (Thibert-Plante & Hendry 2010). Divergence could possibly be too recent to be detected or masked by a population expansion (discussed for cetaceans in ASCOBANS 2007). Classical population genetics may provide information on evolutionary rather than contemporary time scales which are useful for conservation (Pearse & Crandall 2004). Many authors emphasized that integrating ecological data with genetics is essential when trying to determine if populations are demographically independent (Waples *et al.* 2008; Olsen *et al.* 2014). In particular, combining ecological and genetic approaches may be essential for highly mobile and continuously distributed species, such as in the marine environment where defining population structure (i.e. genetic and/or ecological structures) can be challenging (Martien & Taylor 2003; Waples *et al.* 2008). For instance, marine turtles have a complex population structure primarily linked to different migration patterns according to life-stage and sex. Marine turtle specialists do not agree on whether management units should be based on nesting sites, geographical regions or genetic stocks. Thus, Wallace *et al*. (2010) proposed to integrate these three levels. A combination of tools: molecular markers, satellite telemetry and environmental data can provide complementary information on population structure and connectivity for these species (Godley *et al.* 2010). School sharks are an example of a species whose management units

were defined with both genetic and ecological data. While genetics indicated weak or no genetic differentiation between New-Zealand and Australia, tagging data showed low rates of movements between areas. Two management units have thus been defined (see references and personal communications in Waples *et al.* 2008). It should be noted that political and country boundaries, although not biologically meaningful, may also have an important role in management plans and decisions (Waples *et al.* 2008).

Ecological tracers could be an interesting tool to reveal ecological structure (i.e. differences in diet or habitat use). They provide information on population structure over shorter time scales than neutral genetic markers, which could be more relevant for management (see discussion on cetacean population structure in ASCOBANS 2007). For instance, while a single stock of weakfish was defined for the eastern coastal waters of the United States based on genetic results; stable isotopes and trace elements indicated significant population sub-structure and natal homing (Thorrold *et al.* 2001). Thus, Thorrold *et al.* (2001) recommend to take the spatial structure and spawning site fidelity into account in fishery management plans and Marine Protected Area designations.

Understanding social structure can also be relevant for the management of social species. Different social clusters, with distinct habitat use or feeding techniques, can have contrasting foraging success, depending on environmental conditions (e.g. clans of sperm whales during 'El Niño/Southern Oscillation', Whitehead & Rendell 2004), which could affect their reproductive success and fitness. Thus, it may be essential to preserve different social clusters with their own behavioral/cultural traits. In addition, social knowledge and traditions, held by the oldest individuals in some social mammals such as killer whales and elephants, can be altered by poaching (McComb *et al.* 2001; Williams & Lusseau 2006). A disrupted social structure can have negative fitness impact (McComb *et al.* 2001; Gobush *et al.* 2008). Whitehead *et al.* (2004) argued that for some species, such as whales, dolphins and elephants, it is important to preserve cultural variations and that cultural traits should be included in the definition of conservation units. In addition, modeling work showed that it is important to take social structure into account when evaluating the viability of a population as social organization may have an impact on the number of breeders (Vucetich *et al.* 1997).

4) Study model: bottlenose dolphin and research questions

a) Studying cetacean population structure: interest and challenges

Cetaceans are highly mobile mammals which can show various levels of genetic and ecological structures as well as morphological variations both at large and very fine scales (e.g. Sellas *et al.* 2005; Fontaine *et al.* 2007; Viaud-Martinez *et al.* 2008; Foote *et al.* 2009; Ansmann *et al.* 2012b; Wilson *et al.* 2012; de Bruyn *et al.* 2013). They can have complex social structures that vary from solitary individuals in mysticetes, where only mothers and calves form stable bonds (e.g. Valsecchi *et al.* 2002) to stable matriarchal societies for pilot whales and killer whales (Amos *et al.* 1993; Pilot *et al.* 2010). They are therefore particularly suitable models to study social, ecological and genetic structures and their interaction in shaping structuration patterns.

Nevertheless, as they spend most of their time underwater, studying cetacean structuring patterns is particularly challenging. Individual monitoring using the marks on the fins through photo-identification that is described in more details in Chapter 2 enables the study of social structure and demography (Figure 1.1). However, field work is strongly constrained by sea conditions. In addition, while photo-identification monitoring is well suited for coastal areas and relatively small populations, its utility in offshore waters where small cetacean populations are generally large and highly mobile and their distribution largely unknown, is limited.

Figure 1.1. Photo-identification work on bottlenose dolphins.

Therefore, indirect methods to study their ecology and population structure are necessary. Genetic, stable isotope, fatty acid, and pollutant studies can be carried out using samples from biopsied and stranded animals. Samples can be collected from free-ranging animals using a crossbow or a modified rifle that collect both skin and blubber samples (Figure 1.2a, Barrett-Lennard *et al.* 1996; Krützen *et al.* 2002). Several studies reported that the behavioral reactions of cetaceans were limited and only short-term, and no healing complications or infections were reported (e.g. Weller *et al.* 1997; Krützen *et al.* 2002; Tezanos-Pinto & Baker 2011). However, spending considerable time in the field may be needed to achieve a suitable number of samples, and offshore sampling can be difficult and costly. Sampling stranded animals (Figure 1.2b), although having inherent bias such as the uncertainty of the origin of the individuals, can be a cost-effective and non-invasive method of getting samples. As detailed in Chapter 5, drift prediction models can be used to determine the most likely area of death of the individuals, which enhances the power and precision of working with tissue samples from stranded animals (Peltier *et al.* 2012).

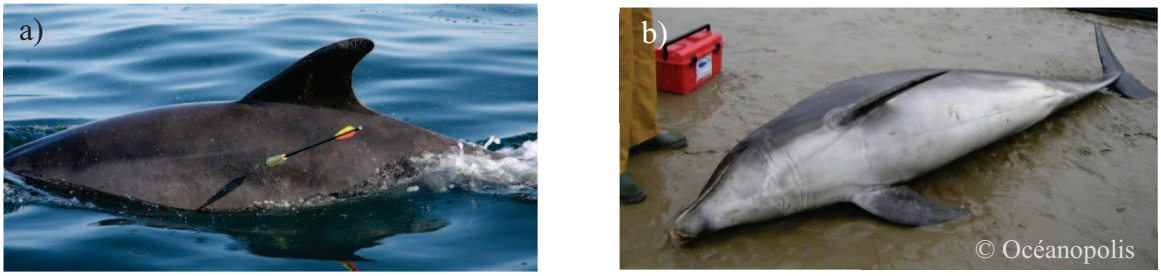


Figure 1.2. a) Biopsy sampling of bottlenose dolphins using a crossbow. b) Stranded bottlenose dolphin.

b) Why studying bottlenose dolphins?

Common bottlenose dolphins, *Tursiops truncatus*, have a worldwide distribution in temperate and tropical waters, in inshore and coastal (including harbors, rivers, estuaries and fiords), deep pelagic and insular waters (Figure 1.3, Leatherwood & Reeves 1990; Wells & Scott 1999; Hammond *et al.* 2012). Their range does not extend to polar waters. The highest northern and southern latitudes where resident communities (i.e. groups of individuals of the same species that co-occur in space and time and have an opportunity to interact with each

other) are found are respectively Scotland (Moray Firth, Wilson *et al.* 1999) and the South Island of New-Zealand (Fiordland, Currey *et al.* 2009a). As they occur across a wide range of habitats potentially facing distinct ecological pressures, they provide an interesting model to investigate the drivers of social and population structures. As detailed above, cetaceans are difficult to access which makes the study of their social and population structures challenging. However, bottlenose dolphins are extensively studied, making comparisons easier, which could help determine the underlying ecological and evolutionary processes driving social and population structures of the species.

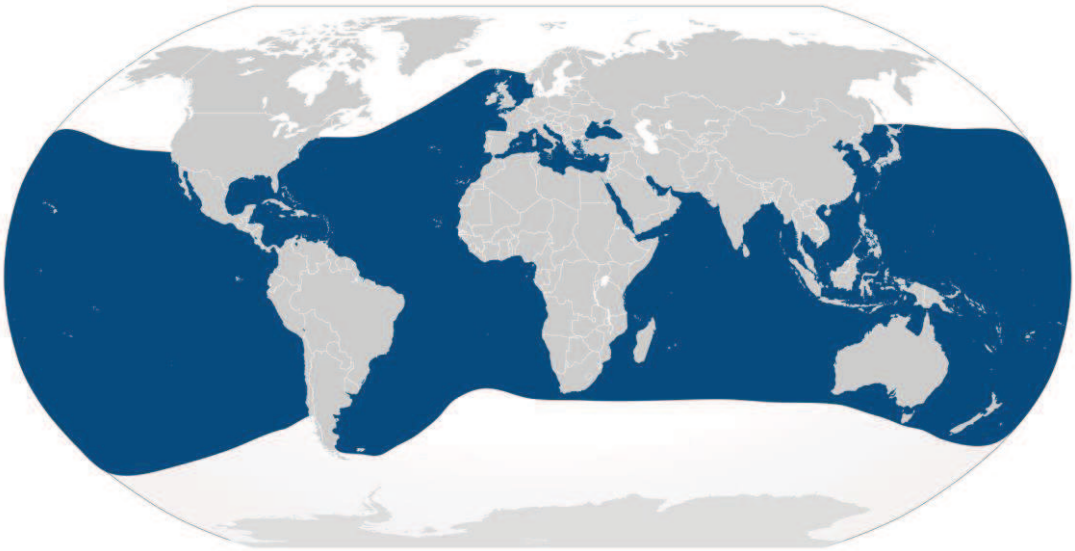


Figure 1.3. Bottlenose dolphin (*Tursiops truncatus*) range distribution, source: iucnredlist.org.

c) Taxonomy and variations in ecology, morphology and genetic structure

The taxonomic status of bottlenose dolphins (*Tursiops* sp.) remains unresolved and the genus is not monophyletic (the taxonomy of Delphininae was recently reviewed in Perrin *et al.* 2013). Two species are recognized: common bottlenose dolphins *Tursiops truncatus* (Montagu 1821) and Indo-Pacific bottlenose dolphins *Tursiops aduncus* (Ehrenberg 1832, LeDuc *et al.* 1999; Wang *et al.* 1999, 2000b, a). While common bottlenose dolphins have a worldwide distribution range (Figure 1.3), Indo-Pacific bottlenose dolphins are only found in

warm temperate to tropical Indo-Pacific areas. A third species has been described in South-East Australia (the Burrunan dolphin, *T. australis*, Charlton-Robb *et al.* 2011) but its validity is debated. A subspecies of common bottlenose dolphin is recognized in the Black Sea, *T. Truncatus ponticus* (Viaud-Martinez *et al.* 2008). Here, we will focus on common bottlenose dolphins, although there are references to both common and Indo-Pacific bottlenose dolphins.

Common bottlenose dolphin feeding ecology and morphology is variable across its distribution range. Two distinct ecotypes, i.e. "coastal" and "pelagic" have been described in the North-West Atlantic (NWA) and in the North-East Pacific (NEP, reviewed in Curry & Smith 1998). We define "pelagic" here as dolphins mainly occurring in deep waters (i.e. deeper than 200 m). The term "pelagic" is interchangeably used with "offshore" in the literature. We choose "pelagic" to refer to individuals occurring in deep-waters, even if they are close to shore (e.g. the Strait of Gibraltar, Spain). We acknowledge that pelagic can also mean "live in the water mass" in contrast to benthic. "Coastal" refers to individuals mainly sighted in shallow waters (less than 200 m, but in majority less than 40 m deep).

In the NWA and the NEP, pelagic and coastal bottlenose dolphins are genetically, ecologically and morphologically distinct and show different parasite loads (Walker 1981; Duffield *et al.* 1983; Hersh & Duffield 1990; Mead & Potter 1995; Curry & Smith 1998; Hoelzel *et al.* 1998b; Walker *et al.* 1999; Segura *et al.* 2006; Kingston *et al.* 2009; Barros *et al.* 2010; Perrin *et al.* 2011). While genetic differentiation is found in both areas, pelagic and coastal ecotypes are monophyletic for mitochondrial DNA only in the NWA (Curry & Smith 1998; Hoelzel *et al.* 1998b; Segura *et al.* 2006; Kingston *et al.* 2009). In the North-East Atlantic (NEA), although ecotype differentiation has been suggested, it was not tested explicitly (e.g. Fernandez *et al.* 2011a; Mirimin *et al.* 2011).

Fine-scale genetic structure is observed in coastal and inshore waters worldwide, presumably as a result of philopatry and habitat/resource specializations (e.g. Sellas *et al.* 2005; Mirimin *et al.* 2011). Although often resident in inshore and coastal areas, large-scale movements have been reported, both in coastal and pelagic waters (Defran *et al.* 1999; Wells *et al.* 1999; Robinson *et al.* 2012).

d) Life-histories and social structure

Bottlenose dolphins can live up to at least 57 years for females and 48 years for males. They reach sexual maturity between 5 to 13 years for females and between 8 and 14 years for males. Calves usually stay from 3 to 5 years with their mother, with separation often coinciding with the birth of the next calf. Gestation period lasts 12 months and inter-birth intervals usually range from 3 to 6 years (reviewed in Wells & Scott 1999; Connor *et al.* 2000). Information on the life-history mainly originates from the well-studied population of Sarasota Bay (coastal ecotype of the NWA) but might vary slightly across the geographical range of the species. Nevertheless, bottlenose dolphins are long-lived animals with a low reproductive rate.

Bottlenose dolphin (*Tursiops* sp.) social structure is defined as fission-fusion, where group composition changes on an hourly or a daily basis. Besides having a majority of short-term associates, individuals can also share some strong and long-term relationships (Connor *et al.* 2000). Group sizes, patterns of relationships within and between sexes, relatedness, and temporal stability of associations can be variable across the wide geographical range of the species (e.g. Connor *et al.* 2000; Krützen *et al.* 2003; Lusseau 2003; Wiszniewski *et al.* 2010b; Augusto *et al.* 2011; Connor *et al.* 2011; Wiszniewski *et al.* 2012a). The most detailed information came from the long-term studies of populations of Australia (Shark Bay, Connor *et al.* 2000) and Florida (Sarasota Bay, Wells *et al.* 1987). Social structure variations will be discussed in more details in Chapters 3 and 4.

e) Bottlenose dolphins in the North-East Atlantic, distribution and conservation status

In the North-East Atlantic, bottlenose dolphins are observed in both coastal and pelagic waters. They can form resident communities of tens to a few hundreds of individuals in bays, estuaries or coastal areas (Figure 1.4., e.g. Liret 2001; López 2003; Pesante *et al.* 2008; Augusto *et al.* 2011; Berrow *et al.* 2012; Cheney *et al.* 2012). Mobile coastal communities have been recorded around Ireland and in the Gulf of Cadiz (O'Brien *et al.* 2009; Giménez *et al.* 2013). Resident individuals are observed in deep waters of the Strait of

Gibraltar and around the Azores, although the majority of individuals are transient around the Azores (>95%, Silva *et al.* 2008; Chico Portillo *et al.* 2011).

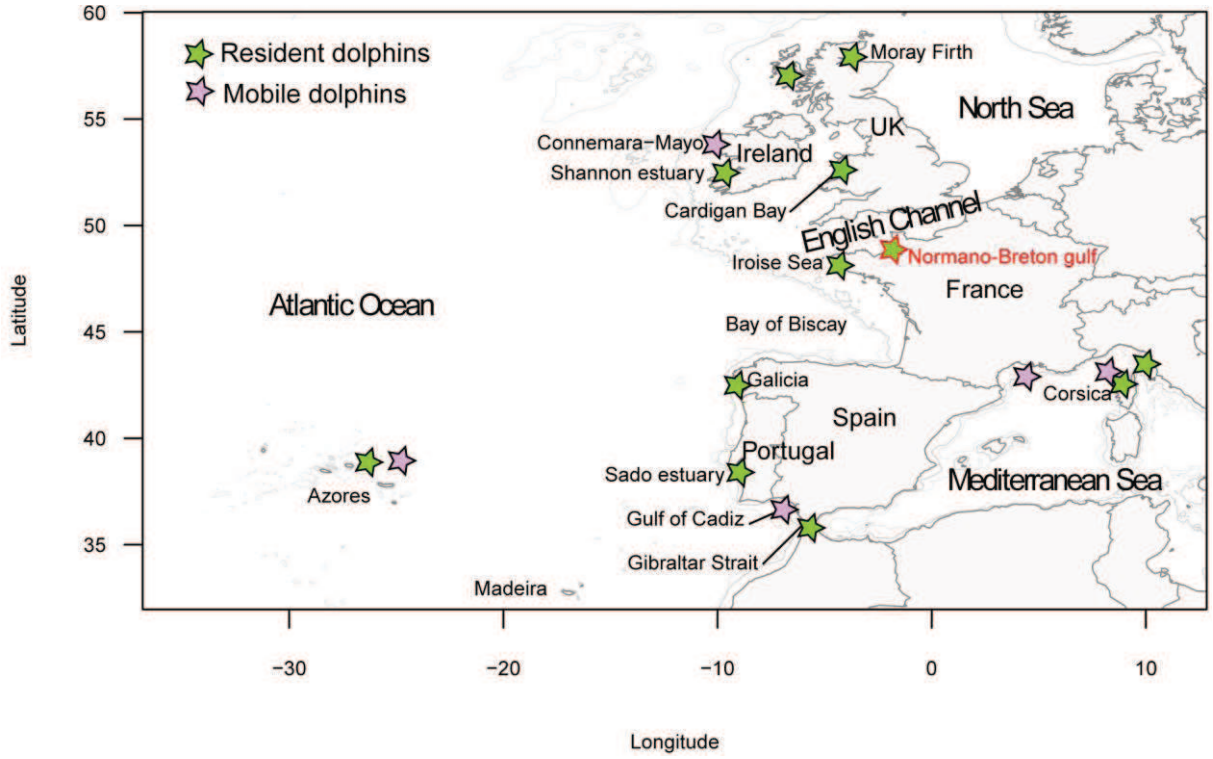


Figure 1.4. Mobile and resident bottlenose dolphin communities inferred using photo-identification data in the North-East Atlantic and the Mediterranean Sea. The list may not be exhaustive. The Normano-Breton gulf (English Channel) population is highlighted in red.

Bottlenose dolphins also occur in pelagic waters in particular along the shelf edge where abundance estimations are tens of thousands of individuals (Figures 1.5a and 1.5b, Certain *et al.* 2008; Hammond *et al.* 2009; Hammond *et al.* 2013).

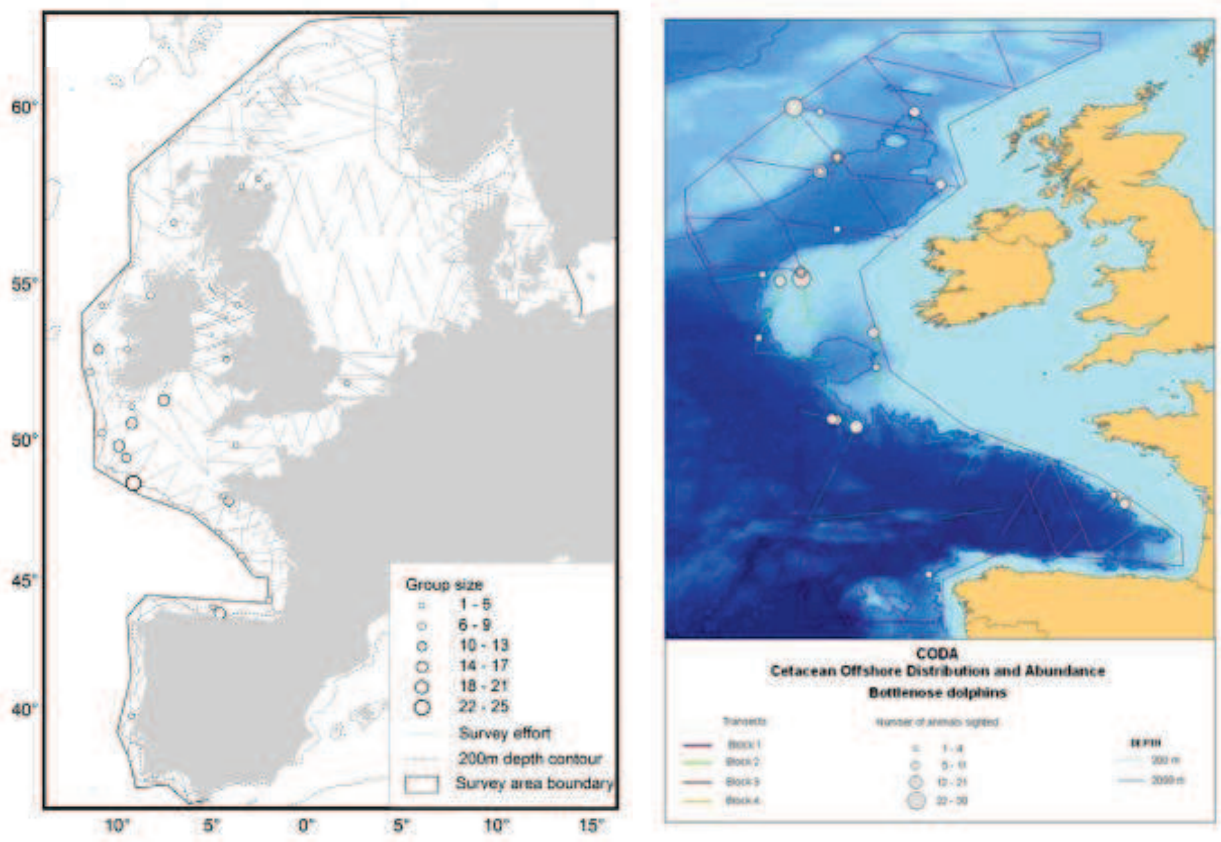


Figure 1.5. Sightings of bottlenose dolphins during a) SCANS-II (Small Cetacean Abundance in the North Sea and Adjacent waters surveys, Hammond *et al.* 2013) and b) CODA (Cetacean Offshore Distribution and Abundance, Hammond *et al.* 2009) surveys.

In the Mediterranean Sea, resident communities are known around Corsica and North-West Italy. A few mobile individuals were reported between Corsica and France and along the North-West coast of Italy (Gnone *et al.* 2011). Along the Mediterranean coast of France, individuals are relatively mobile (Labach *et al.* 2012). Overall, sightings were concentrated in deep-water (>200 m) areas during winter aerial surveys (SAMM, Suivi Aérien de la Mégafaune Marine, 2011/2012, E. Pettex, personal communication). Global abundance estimation is several thousands of individuals for the Mediterranean Sea (Forcada *et al.* 2004; Bearzi *et al.* 2008; Gnone *et al.* 2011).

Bottlenose dolphins (*T. truncatus*) are listed under Annex II of the CITES convention (Convention on International Trade in Endangered Species of Wild Fauna and Flora), which includes species that are not necessarily threatened with extinction but may become vulnerable if trade is not controlled. They are globally considered as "least concerned" by the IUCN (International Union for Conservation of Nature) Red List of Threatened Species (Hammond *et al.* 2012). Populations in the Mediterranean Sea and Black Sea (*T. truncatus ponticus* for the latter) are listed as respectively "vulnerable" and "endangered" (Bearzi *et al.* 2012; Birkun 2012).

Bottlenose dolphins are protected in European waters by Habitats Directive (92/43/22C). They are listed in Annex II as a species whose conservation requires the creation of Special Areas of Conservation and in Annex IV as in need of strict protection. As human activities are increasing in both coastal and pelagic waters, potential threats include pollutants (the species show high levels of PCBs, e.g. Méndez-Fernandez *et al.* 2014), noise pollution in particular for constructions (e.g. Pirotta *et al.* 2013), disturbance by tourism activities (no studies have yet been conducted in Europe, but see Steckenreuter *et al.* 2012 for Australia) and bycatch (e.g. Morizur *et al.* 1999; López *et al.* 2003; Rogan & Mackey 2007). Studying population structure is therefore crucial for defining management plans and conservation units.

f) Research questions

An important community of bottlenose dolphins is present in the Normano-Breton gulf (English Channel, Figure 1.4), as suggested from opportunistic sightings and photo-identification work. However, no dedicated study has yet been conducted on this community. It is important to gather knowledge on the abundance, and social and population structures of this bottlenose dolphin community, to ensure effective protection and management plans are in place. First, it is essential to set up a demographic monitoring plan for these dolphins, especially as human activities will increase in the upcoming years (e.g. there are projects of offshore water and wind turbines in the area). As detailed earlier, determining the structure of the community in terms of social, ecological and genetic structures is also important for management purposes. In addition, by using a combination of approaches, a better understanding of the factors driving sociality can be determined. Although being fission-

fusion societies, bottlenose dolphins show variations in social structure characteristics (for example in stability and strength of associations, influence of relatedness or group sizes) across the large range of habitats where they occur. Thus, a comparison with other communities in the world will help to unravel the evolutionary and ecological processes that may shape the social organization of bottlenose dolphins and more broadly other social mammal species.

To evaluate whether bottlenose dolphins of the Normano-Breton gulf were genetically isolated, it is important to place them on a wider context. Although bottlenose dolphin genetic structure has been studied locally in the North-East Atlantic (Quérouil *et al.* 2007; Fernandez *et al.* 2011b; Mirimin *et al.* 2011) as well as at a larger scale (but with relatively small sample sizes, Natoli *et al.* 2005), a global understanding of their genetic structure is lacking. In addition, we do not known if they form two ecotypes. Hence, another objective of this study is to evaluate the genetic structure of bottlenose dolphins in the North-East Atlantic using samples from both coastal and pelagic waters covering an unprecedented large area. Then, we aim to investigate how population structure and the formation of the coastal and pelagic ecotypes were triggered using past demographic history analyses. A comparison of the ecology and morphology of bottlenose dolphins of the two ecotypes was carried out to aid our understanding on how ecotype differentiation is maintained. More generally, this study is aimed at contributing to a better knowledge on the evolutionary and ecological processes that led to genetic and morphological divergences in highly mobile top predators.

The general objective of my PhD work is therefore to describe and understand the fine-scale social and population (i.e. genetic and ecological) structures of bottlenose dolphins in the Normano-Breton gulf and their drivers, as well as the large-scale population structure of the species in the North-East Atlantic.

g) Manuscript organization

I give a general background on the methods used in Chapter 2.

Chapters 3 and 5 describe bottlenose dolphin social structure in the Normano-Breton gulf (English Channel) and the population structure in the North-East Atlantic respectively. In Chapters 4 and 6, I investigate the possible mechanisms creating and maintaining the described structures.

More precisely, I first focus on bottlenose dolphins in the Normano-Breton gulf, English Channel, in Chapters 3 and 4 (see general location of the area in Figure 1.4, Figures 1.6a and 1.6b).

In Chapter 3, social structure is described and abundance is estimated.

Then in Chapter 4, I test whether the social clusters identified in Chapter 3 correspond to genetic and ecological clusters. I also evaluate the relative influence of relatedness, gender and ecology on association patterns and discuss the possible drivers of sociality.

Figure 1.6. a) juvenile and b) adult male coastal bottlenose dolphins in the English Channel.

Chapter 5 presents results from the first study to evaluate the genetic structure of bottlenose dolphins in both coastal and pelagic waters in the whole North-East Atlantic.

Migration rates and effective population sizes* were also estimated. I discuss hypotheses that might explain this structure.

Then in Chapter 6, the most likely population history of bottlenose dolphins in the North-East Atlantic is investigated to test if divergence between populations were triggered by past environmental changes. The ecology and morphology of bottlenose dolphins from the coastal and pelagic ecotypes, identified in Chapter 5, are characterized. I discuss how genetic and morphological divergences may be created and maintained in mobile social species.

These chapters correspond to publications that are accepted, submitted or to be submitted. To avoid repetition, article material and methods have been slightly edited. As there are several co-authors, I used "we" in these chapters and I highlight below my personal contribution to each of the chapters.

In Chapter 7, I synthetize the findings and discuss the results in a broader context, in particular the interaction between sociality, ecology and genetics, the interest of a multi-disciplinary approach to define the structure of populations as well as management implications. I finish with proposing new perspectives of research.

Publications included and personal contribution

Chapter 3

Louis M., Gally F., Barbraud C., Béesau J., Tixier P., Simon-Bouhet B., Le Rest K. and Guinet C. *submitted*. Social structure and abundance of coastal bottlenose dolphins, *Tursiops truncatus*, in the Normano-Breton gulf, English Channel.

I took part in the field work during my PhD from April to October 2011 and before my PhD during July and August 2009 and from July to December 2010. I did the majority of the photo-identification work (75%), the remaining was done with the help of GECC (Groupe d'Etude des Cétacés du Cotentin lead by F. Gally) volunteers. I double-checked all the identifications. I performed all the statistical analyses with advice from some of my co-authors. I wrote the paper and my co-authors commented on the manuscript.

Chapter 4

Louis M., Simon-Bouhet B., Viricel A., Lucas T., Gally F., Cherel Y., Guinet C. *to be submitted.* Evaluating the influence of ecology, kinship and phylogeography on the social structure of resident coastal bottlenose dolphins.

I organized the biopsy sampling field work. I took part in most of the biopsy sample collection (85%) where I did either the biopsy sampling, took the photos of the sampled individuals or drove the boat. The DNA extraction, optimization of the microsatellite markers, the molecular sexing, and the amplification of a portion of the mitochondrial DNA were carried out by a master student, Tamara Lucas, and by myself. I did the microsatellite genotyping for 20 microsatellites on a LICOR DNA analyzer while the individuals were amplified for 7 microsatellites by a private society, Genoscreen on an ABI DNA sequencer. I did all the scorings. I did the stable isotope lab work, the statistical analyses and I wrote the manuscript. Co-authors gave me advice on statistical analyses or on the manuscript and contributed to the design of the study.

Chapter 5

Louis M., Viricel A., Lucas T., Peltier H., Alfonsi E., Berrow S., Brownlow A., Covelo P., Dabin W., Deaville R., de Stephanis R., Gally F., Gauffier P., Penrose R., Silva M. A., Guinet C. and Simon-Bouhet B. 2014. Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic. *Molecular Ecology*, 23: 857-874.

I did not take part in the sample collection, apart from the ones from the Normano-Breton gulf mentioned previously. They were collected by the organizations of each collaborator. I contacted them for collaboration at the beginning of the PhD and centralized the samples. As mentioned earlier, part of the DNA extraction, molecular sexing and amplification of the mitochondrial control region were done with the help of a master student. Microsatellite genotyping was performed as mentioned for the previous chapter. I did all the statistical analyses, apart from the drift modeling which was done by Hélène Peltier and I wrote the manuscript. Co-authors gave me advice on statistical analyses, commented on the manuscript or provided tissue samples.

Chapter 6

Louis M., Fontaine M., Spitz J., Schlund E., Dabin W., Deaville R., Caurant F., Cherel Y., Guinet C. and Simon-Bouhet B. *to be submitted*. Ecological opportunities and specializations shaped genetic divergence in a highly mobile marine top predator.

Genetic data from Chapter 5 were used. I did the stable isotopes lab work and statistical analyses. Morphometric measurements were recorded by the french Stranding Network. Part of the morphometric analyses were performed by a master student, Erika Schlund. Jérome Spitz did the stomach content lab work and analyses, and wrote the methods and results of this section. Population history analyses were done by Michael Fontaine, he wrote the methods and the results of these analyses. I learned how to carry out the population history analyses and wrote the rest of the manuscript. Co-authors provided samples or advices on statistical analyses or commented the manuscript.

CHAPTER

2

METHODOLOGICAL BACKGROUND

This chapter aims at describing the general characteristics, principles or assumptions of the different methods used in this PhD that will not be detailed in the material and methods of each article chapter. This background is however important to understand the analyses that have been carried out.

1) A combination of approaches: from recent to evolutionary time scales

Different approaches were used to study bottlenose dolphin population and social structures. This chapter gives an overview of the methods used and some of their applications. The different approaches inform us on different time scales that are summarized in Figure 2.1.

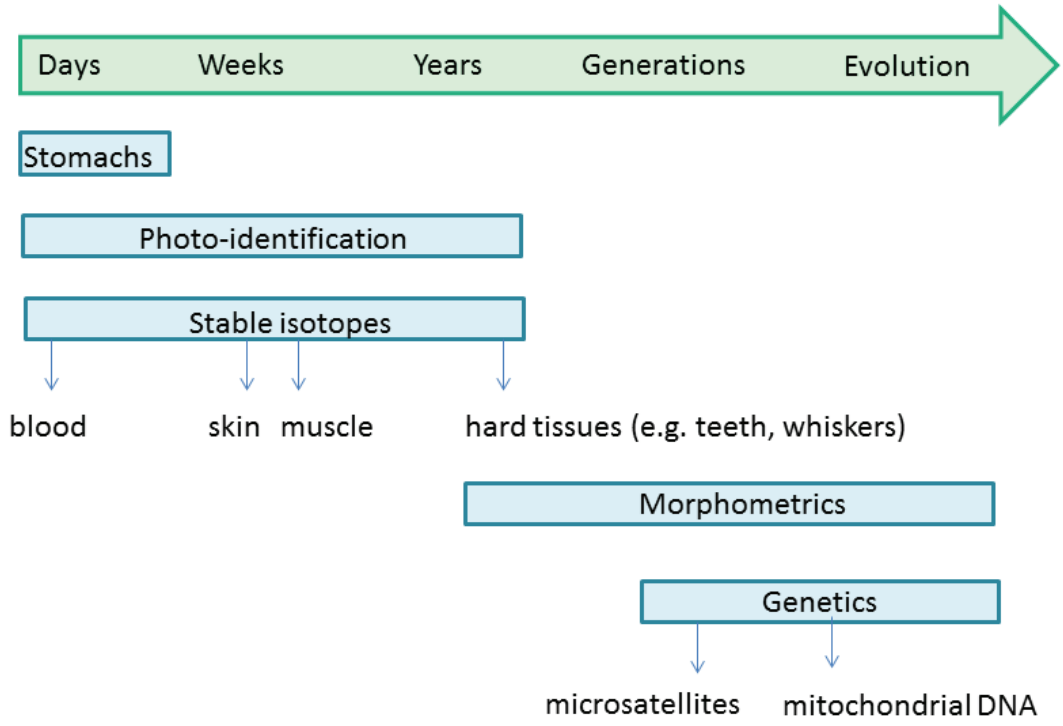


Figure 2.1. Time scales covered by the methods used in the PhD.

a) Photo-identification

Individuals are identified thanks to natural distinctive physical features such as nicks, scars and coloration patterns. The method has been used to recognize individuals in a wide range of cetacean species and the part of the body used for identification can vary (Hammond *et al.* 1990; Würsig & Jefferson 1990). For example, many dolphin species are identified using the marks and nicks on their dorsal fins (Figure 2.2). Sperm whales or humpback whales are mainly recognized by examining the characteristics of their flukes. Pigmentation comparisons allow to individually identified blue whales, bowhead whales or fin whales. Photo-identification is not limited to cetaceans. For instance, seals, giraffes, zebras, tigers among other species can be individually recognized thanks to their coloration patterns.

Figure 2.2. Photo-identification matching of bottlenose dolphins using nicks and marks on the dorsal fin and the upper back.

A catalogue is build and used to re-identify individuals on the photos taken during each field trip (Figure 2.2). When an animal is not recognized, he is added to the catalogue as

a new individual. It is important to grade the level of marking of each individual and the quality of the photos to avoid misidentifications. This point will be detailed in Chapter 3.

Photo-identification can provide information on association patterns, demography, habitat use or behavior from very short-time scales (days) to the life-span of the individuals if the work is carried out on a long-term basis.

A limitation of this method is that it is constrained by the spatio-temporal coverage of the field work.

Photo-identification can be used for mark-recapture studies to estimate abundance and survival, similarly as artificial marking like bird ringing (Clobert *et al.* 1987; Wilson *et al.* 1999; Currey *et al.* 2009b). The first capture (identification) is followed by several sampling occasions where the individual is recaptured (re-identified) or not. The succession of presence and absence in each sampling occasion represents the capture history of each individual. Mark-recapture models are applied on these capture stories. Using the number of marked individuals and their proportion in each sampling occasion, demographic parameters such as abundance, survival or growth rate can be estimated (Lebreton *et al.* 1992; Schwarz & Seber 1999; Amstrup *et al.* 2005). Depending on the characteristic of the population ("closed" that is with no death, birth and migration or "open") and the parameters to estimate, different models are chosen (Amstrup *et al.* 2005). Abundance estimation for a "closed" population of bottlenose dolphins in the Normano-Breton gulf, English Channel, during summer is detailed in Chapter 3.

Using photo-identification, it is also possible to work on movements patterns. For highly mobile animals, it often requires a collaborative framework and sharing of photo-identification catalogues among organizations worldwide. In Europe, bottlenose dolphin movements have been reported between Scotland and Ireland thanks to photo-identification (Robinson *et al.* 2012). On a larger scale, humpback whales have been re-sighted between Australia and Antarctica or Cape Verde and Iceland (Jann *et al.* 2003; Rock *et al.* 2006).

Social structure analyses have been applied on photo-identification data in a wide range of taxa, e.g. dolphins (Lusseau *et al.* 2003), giraffes (Carter *et al.* 2013), kangaroos (Best *et al.* 2013) or black-tip reef sharks (Mourier *et al.* 2012). The assumptions of these

analyses are that physical proximity, that is being member of the same group, indicates social affiliation and that the time spent together is correlated with the strength of social affiliation (Bejder *et al.* 1998). Individuals observed in the same social group are considered associated. The calculation of association indices between pairs of individuals is the basis of social structure analyses (see Whitehead 2008a for an exhaustive description of the methods). These analyses are detailed in Chapter 3 where bottlenose dolphin photo-identification data collected in the Normano-Breton gulf, English Channel, are used to describe social structure.

b) Ecological and diet indicators

Stomach contents

Stomach content examination can inform on the diet of an individual at the species level as well as on the characteristics (length and mass) of individual prey using allometric relationships based on hard tissues like otoliths. Stomach contents indicate the diet of an animal over the last few days. One major limitation of this technique is the digestion rate that can vary for different prey species. In particular, some species can be overestimated because of the persistence of their hard pieces in the stomachs as they are difficult to digest (Santos *et al.* 2001a). In addition, analyses can only be performed on dead animals.

The stomach contents of stranded animals have been used to study the diet of numerous cetacean species (e.g. Santos *et al.* 1999; Spitz *et al.* 2006). There is some uncertainty if stomach contents of stranded animals are representative of the diet of alive wild individuals. The physical condition of individuals may indeed affect their foraging capacities and some classes such as young or old individuals may eventually be over-represented in strandings. However, in Florida, results on prey species composition obtained using stomach content analyses on stranded animals and using molecular identification of prey in feces and gastric samples of free-ranging dolphins were highly consistent (Dunshea *et al.* 2013). In this dissertation, stomach contents are used, in complement with stable isotope analyses, to understand the foraging ecology of bottlenose dolphins in Chapter 6.

Stable isotopes

In the environment, natural elements can be found in different isotopic forms. Isotopes of any given chemical element have different number of neutrons, thus their atomic mass is different. Therefore, in biogeochemical reactions, the heavy isotopes accumulate in substrates as they react slower than light isotopes while products are depleted in heavy isotopes (Figure 2.3). This process, called the isotopic fractionation, controls isotope distribution (ratio of heavy to light isotopes) in the environment (Fry 2006).

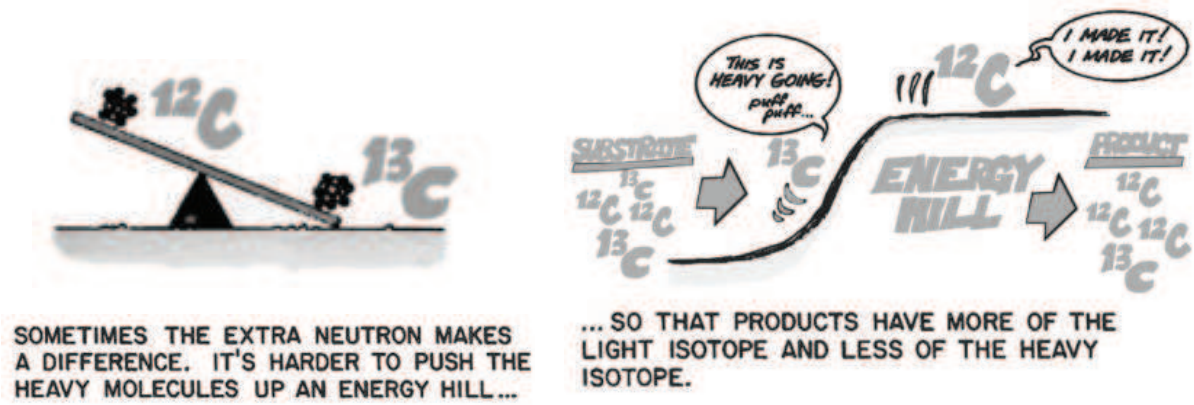


Figure 2.3. Illustration of the isotopic fractionation process (source: Fry 2006).

In ecology, stable isotope analyses are indirect tools to study foraging ecology. There are based on the principle "you are what you eat", that is the biochemical composition of the tissue of a consumer is linked to the one of its prey (Kelly 2000). $\delta^{13}C$ ($^{13}C/^{12}C$) and $\delta^{34}S$ ($^{34}S/^{32}S$) vary according to primary producers. In the marine environment, $\delta^{13}C$ and $\delta^{34}S$ indicate consumer foraging habitats such as inshore *vs* offshore or pelagic *vs* benthic habitats. $\delta^{13}C$ also vary along latitudinal gradients (Peterson & Fry 1987; Kelly 2000; Connolly *et al.* 2004). $\delta^{34}S$ do not vary between consumers and prey and $\delta^{13}C$ vary little with increasing trophic level (generally less than 1 ‰, see review in Peterson & Fry 1987). In contrast, ^{15}N is preferentially accumulated in the tissues of the consumers relative to their diet, therefore an average enrichment of 3 to 4 ‰ in $\delta^{15}N$ ($^{15}N/^{14}N$) is generally observed with each increasing trophic level (see review in Kelly 2000). $\delta^{15}N$ is therefore used as an indicator of trophic

position. It can also reflect feeding areas in some ecosystems (e.g. inshore *vs* offshore in the Bay of Biscay, Chouvelon *et al.* 2012).

The turn-over rate of stable isotopes in a given tissue depends on the tissue metabolic rate. Therefore, stable isotopes are integrated over different time scales in different tissues (Tieszen *et al.* 1983; Hobson & Clark 1992). For example, in plasma, stable isotopes will inform on the diet and habitat use during the last few days preceding the tissue sampling (e.g. Podlesak *et al.* 2005) and in skin or muscle during several weeks to months (e.g. Tieszen *et al.* 1983; Browning *et al.* 2014). In hard tissues, like teeth, bones, whiskers or baleen plates, stable isotopes are integrated over the entire life of the individuals (e.g. Best & Schell 1996; Estrada *et al.* 2006; Mendes *et al.* 2007; Kernaleguen *et al.* 2012). The integration time of a specific soft tissue can also vary according to the species considered as metabolic rates are also species-specific (MacAvoy *et al.* 2006). One drawback of this method is that interpretation might be difficult especially if the baseline values of the ecosystems are not known (reviewed in Ramos & Gonzalez-Solis 2012). For instance, similar stable isotope signatures could be the result of a similar diet in the same habitat or a dissimilar diet in distinct habitats that have the same baseline values.

Stable isotopes have numerous applications in ecology and environment studies. To cite only a few examples, stable isotopes have been used to identified foraging habitats and migration patterns in a wide range of taxa (i.e. insects, fish, birds or mammals, see review in Rubenstein & Hobson 2004). By comparing stable isotopes in consumers and potential prey, or applying stable isotope mixing models on predator and prey data, it is possible to estimate the diet of a predator (e.g. Cherel *et al.* 2008; Huckstadt *et al.* 2012; Watt *et al.* 2013). As stable isotopes reflect habitat use and diet composition, stable isotope analyses can also help to determine population structure (e.g. Rooker *et al.* 2008a; Olin *et al.* 2012; Rioux *et al.* 2012; Wilson *et al.* 2012). Stable isotope signatures could be used as proxies of ecological niches (Newsome *et al.* 2007; Jackson *et al.* 2011). The ecological niche has been defined by Hutchinson (1957, 1978) as an *n*-dimensional hyper-volume with biotic and abiotic environment and resource variables as axes. These axes may be quantified by stable isotope signatures of $\delta^{15}N$ and $\delta^{13}C$ (or others such as $\delta^{34}S$) as they inform on either or both trophic level and environment and resource uses (Bearhop *et al.* 2004; Newsome *et al.* 2007; Jackson *et al.* 2011). Although the limits of stable isotope analyses should be recognized (i.e. see

above and complex physiological processes may influence stable isotope tissue composition), isotopic niches can therefore be used to investigate ecological niches (Newsome *et al.* 2007).

In Chapters 4 and 6 stable isotopes are used as indicators of foraging ecology and habitat use (i.e. ecological niches) as well as tools to investigate population structure.

c) Morphometrics

Morphometrics is the quantitative analysis of the size and/or the shape of an organism. They can be used, together with other morphological characters (e.g. coloration patterns) and genetic analyses, to separate species (e.g. short-finned and long-finned pilot whales are distinguished with the ratio of the length of the pectoral fin to the total length of the body along with the number of teeth per half jaw, Van Bree 1971; Robineau 2005). They can also be used in evolutionary ecology studies to understand how environmental conditions might influence morphological traits such as body size, size of appendices or cranial traits on short to evolutionary time scales (e.g. Grant & Grant 2002; Viaud-Martinez *et al.* 2007; Berner *et al.* 2010; Rode *et al.* 2010). For instance, body length can strongly be constrained by environmental conditions. Decreased body length in a polar bear population over two decades was correlated with a decline in sea-ice habitat availability (Rode *et al.* 2010). A rapid increase in body length in a population of fur seals may be the result of selective processes, in particular as bigger individuals have higher reproductive success (Authier *et al.* 2011). Resource polymorphism may also shape morphological traits (Smith & Skúlason 1996). The shape and size of beaks of darwin's finshes varied across years probably because of variations in the availability of their food, i.e. seeds of different sizes, and the presence of competitors (e.g. Grant & Grant 2002, 2006). Although morphological trait evolutions for the latter examples were rapid, morphological divergence may be constrained by time. For example, Canada lake and stream threespine sticklebacks, that originated thousands years ago, are highly morphologically differentiated. In contrast, European lake and stream individuals were weakly morphologically distinct, possibly as a result of time constraints on divergence, as they originated less than 150 years ago. Nevertheless, at least some traits have evolved on a

contemporary basis (Berner *et al.* 2010). In cetaceans, morphological variations are observed for example between open oceans and enclosed seas such as "dwarfism" for bottlenose dolphins and harbor porpoises in the Black Sea (Perrin 1984; Viaud-Martinez *et al.* 2007; Viaud-Martinez *et al.* 2008), which may have evolved on an evolutionary time scale. Thus, variations in morphological characters may reveal adaptations to different resource use both in terms of habitats and diets, and can therefore be an indicator of population structure (Perrin 1984). For instance, offshore and coastal bottlenose dolphin ecotypes in the North-East Pacific and in the North-West Atlantic differ in skull features (Hoelzel *et al.* 1998b; Perrin *et al.* 2011). In addition, apical tooth wear differ between weakly genetically differentiated killer whale specialists and generalists in the North-East Atlantic (e.g. Foote *et al.* 2009).

In Chapter 6, morphometric analyses are carried out to characterize bottlenose dolphin ecotypes in the North-East Atlantic.

d) Molecular markers: mitochondrial DNA and microsatellites

Mitochondrial DNA

Mitochondrial DNA is a small circular molecule which is present in numerous copies in animal cells. It is haploid and mostly maternally inherited although heteroplasmic individuals (i.e. for which mitochondrial DNA was biparentally inherited) can be observed in different proportions in some taxa (e.g. Zouros *et al.* 1994; Vollmer *et al.* 2011). As it is haploid, there is generally no recombination (but see Eyre-Walker 2000; Ujvari *et al.* 2007). Evolution rate is five to ten times faster than nuclear DNA in mammals (Moritz *et al.* 1987), with an average mutation rate of 1×10^{-8} per site per year, making it useful in population genetics and phylogenetic studies. Mitochondrial DNA is composed by different regions which have different evolution rates including the control region which is the most variable and rapidly evolving part and thus of interest for population genetic studies. Estimates of mutation rates for the control region of cetaceans vary from 0.5×10^{-8} to 1.3×10^{-6} per site per year (Hoelzel *et al.* 1991; Harlin *et al.* 2003; Alter & Palumbi 2009; Fontaine *et al.* 2010).

As it is haploid and maternally inherited, effective population size at mitochondrial loci is four times lower than at nuclear loci. Mitochondrial genome is therefore more sensitive

to genetic drift and integrates demographic events like population expansions or bottlenecks* since a longer time than nuclear markers.

Polymorphism in the sequence is detected through sequencing. Each haplotype is a unique sequence. Different haplotypes differ by one or more nucleotides because of substitutions, deletions or insertions.

Microsatellites

Microsatellites are nuclear non-coding markers that are bi-parentally inherited and supposedly neutral (i.e. not affected by selection). Also known as "Short Tandem Repeat", they are tandemly repeated sequences where the repeated unit contains typically two to four nucleotides. The number of repeated units at a given locus can differ, resulting in alleles of different sizes. These alleles of different sizes can be separated using electrophoresis.

Microsatellites are highly variable and polymorphic. Mutation rates are higher than in the rest of the nuclear genome, they range from 10^{-5} to 10^{-3} per locus per generation (Crawford & Cuthbertson 1996; Brinkmann *et al.* 1998; Estoup & Angers 1998). They are therefore well-suited for fine-scale genetic structure studies and for investigating recent gene flow.

Combination of the two markers

The different rates of evolution of mitochondrial DNA and microsatellites provide information on processes occurring at different time scales (i.e. on recent processes for microsatellites and on more historical processes for mitochondrial DNA). In addition, the different modes of inheritance can reveal distinct dispersal patterns between males and females (Pardini *et al.* 2001; Bowen *et al.* 2005). Comparisons of the results obtained using the two types of markers can also help to avoid misinterpretations. For instance, as there is generally no recombination for mitochondrial DNA genome, if a mutation is selected, it will impact the whole mitochondrial genome. This phenomenon called selective sweeps can lead to a loss of diversity (Bazin *et al.* 2006) similar to what can be observed for a bottleneck or a

founder event*. If microsatellites also indicate a low level of diversity, the selective sweeps hypothesis would be less supported than the bottleneck or the founder event.

The combination of markers is therefore essential to understand population structure.

Both markers have been extensively used in population genetic studies. Mitochondrial DNA is also largely employed to investigate phylogeny and microsatellites are used to estimate relatedness between individuals and to carry out parentage analyses. In this dissertation, both mitochondrial DNA and microsatellites are used to infer population structure and evolutionary history of bottlenose dolphins in the North-East Atlantic (Chapters 5 and 6) and in the English Channel (Chapter 4). Microsatellites are also used to estimate relatedness between bottlenose dolphins in Chapter 4.

2) Statistical analyses of molecular markers

As my dissertation contains a large part of genetic analyses, I give here a general description of the key statistical methods used and their assumptions to make the reading of the following chapters easier. I do not aim to provide an exhaustive review of all available methods, but the basic principles of the two main analyses used in the following chapters are explained. I first describe the general principle of Bayesian statistics, which were later used both to infer population structure and demographic history. Then, I focus on the detection of genetic structure methods which were employed to infer bottlenose dolphin genetic structure in Chapters 4 and 5. Lastly, I introduce coalescent theory on which demographic history reconstructions are based and the method used in Chapter 6 to infer population history of bottlenose dolphins in the North-East Atlantic: Approximate Bayesian Computation. The details of the methods (such as parameter values) will be given in the material and methods of each chapter.

a) Bayesian statistics

In Bayesian statistics, prior knowledge on the parameters of the model of interest (i.e. the hypothesis to test) is summarized in the prior probability distribution (or prior). Bayes' Theorem produces a posterior probability distribution using the prior and the likelihood of the data given the model. Like *P*-values, posterior probability distributions are a measure of the confidence of a model or of parameter estimates.

The posterior probability distribution $P(\theta|Y)$, that is the probability of the parameters of the model (θ) given the data (Y) is estimated using the Bayes Theorem following the formulation:

$$P(\theta|Y) = \frac{P(Y|\theta)P(\theta)}{P(Y)}$$

where $P(Y|\theta)$ is the likelihood of the data given the parameters of the model, $P(\theta)$ is the prior probability distribution and $P(Y)$ a normalizing constant.

Priors can either be informative or uninformative. The computation of the posterior probability distribution is often performed using Markov Chain Monte Carlo (MCMC). Similarly to Akaike Information Criterion (AIC), the Deviance Information Criterion (DIC) can be used for model comparisons.

b) Genetic structure

Until the end of the 20th century, genetic structure was inferred by defining *a priori* groups of individuals based on geographical, ecological or other characters and by estimating *F*-statistics or conducting analysis of molecular variance to measure the divergence among these predefined groups (Wright 1951; Excoffier *et al.* 1992). This method had several drawbacks. First, groups created *a priori* can be biologically irrelevant, subjective and spurious. In addition, cryptic patterns of genetic structure such as genetic structure with no obvious barrier to gene flow or secondary contact among previously isolated populations cannot be detected. Therefore, Bayesian clustering methods that are based solely on the multilocus genotypes of the individuals have been developed (Pritchard *et al.* 2000). They are

thus objective methods. In addition, the Bayesian framework enables to include spatial information as *a priori* (Guillot *et al.* 2005; Chen *et al.* 2007; Durand *et al.* 2009b).

We used Bayesian clustering methods based on multilocus genotypes to infer the most likely number of populations and assign individuals probabilistically to each population. We used a non-spatial method implemented in STRUCTURE (Pritchard *et al.* 2000; Falush *et al.* 2003) and a method that uses the geographical coordinates of individuals as *a priori* information (software TESS, Chen *et al.* 2007; Durand *et al.* 2009b).

The general principle is (i) to estimate the most likely number of populations and (ii) assign individuals probabilistically to each population.

Bayesian clustering methods

STRUCTURE

In the STRUCTURE model, there are *K* populations (where *K* can be unknown) which are characterized by a set of allele frequencies at each locus. The model considers that the populations are in Hardy-Weinberg Equilibrium* (HWE) and that there is complete linkage equilibrium* between loci within each population. The population structure is defined by minimizing Hardy-Weinberg and linkage disequilibria. The model aims at simultaneously assigning individuals to populations and estimating allele frequencies. A Bayesian approach is used to estimate the parameters of interest that are the number of populations, the populations of origin of each individual and allele frequencies. The Bayesian framework enables to consider the inherent uncertainty of the parameters and to include *a priori* information. In STRUCTURE, the null distribution corresponds to an equal probability for the individuals to be part of each population. Posterior estimates of the parameters are inferred using a MCMC method.

In STRUCTURE, the most likely number of populations (*K*) is *ad hoc* estimated. It is a fixed parameter of the model and several simulations of each *K* values to test should be performed (e.g. 10 simulations for each *K* values from 1 to 10). The number of *K* to test is chosen according to sampling characteristics and the biology of the species. The model choice criterion to estimate the most likely number of populations is the posterior probability of the

data for a given K, $Pr(X|K)$ that is noted $Ln\ P(D)$. This criterion is obtained by first calculating at each step of the MCMC the log likelihood of the data. Then, the mean of the latter values is calculated and half of their variance is subtracted to obtain $Ln\ P(D)$. The value of K for which the maximum $Ln\ P(D)$ is obtained is considered as an indication of the most likely number of populations.

However, using simulations, Evanno (2005) showed that even when the true K value was reached, $Ln\ P(D)$ could form a plateau or could still increase slightly. They proposed another criterion to choose the most likely number of clusters, ΔK. It is an *ad hoc* quantity based on the second order of change of the log probability of the data according to the number of K. They showed, using simulations that it was a good predictor of the "true" number of populations even for complex patterns of population structure. In particular, the method is efficient at detecting hierarchical structure. Both of the described criterion as well as the plots of the individual assignments to each population should be examined in practice (Evanno *et al.* 2005).

Different models have been implemented: without and with admixture and with correlated and uncorrelated allele frequencies. In the model with no admixture, each individual is assigned to one population. The probability that an individual is part of each population can be called "assignment probability" and reveal the uncertainty of the classification. In the model with admixture, it is not the individual itself but fractions of its genome (i.e. allele copy that is "an allele carried at a particular locus by a particular individual") that are assigned to a population. The percentages of the genome of an individual that came from each population are called "admixture proportions" (Figure 2.4.).

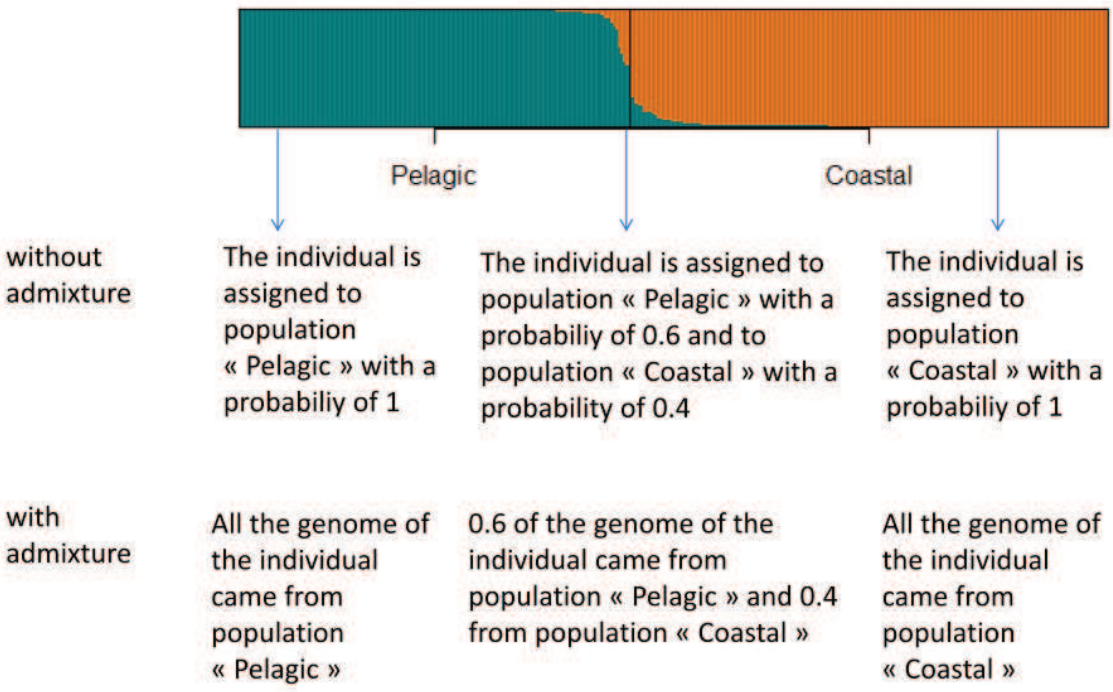


Figure 2.4. How to read a STRUCTURE barplot for the model without admixture and with admixture? Each vertical line on the x axis represents an individual, the y axis represents the assignment probabilities or admixture proportions.

In practice, if each population is thought to be completely discrete, the model without admixture is suitable. However, admixture between populations is relatively common in the field, and sampled individuals could have recent ancestors from several populations. The admixture model is thus often more appropriate.

The model of uncorrelated allele frequencies can be used for populations that are not closely related. In this model, different populations are not expected to have similar allele frequencies, thus subpopulations that share similar frequencies might be merged. The alternative model allows allele frequencies to be correlated when populations are supposed to be closely related due to shared ancestry. It has greater power to detect distinct populations when they are closely related and in the opposite situation (absence or low level or correlations), it will led to similar results than the uncorrelated allele frequency model. It is therefore recommended to use this conservative approach.

For cetaceans which are highly mobile, when individuals are continuously distributed or when discrete groups of individuals are still geographically close to another, the admixture models with correlated allele frequencies seem to be an appropriate model.

Although not detailed here, latter developments included methods that allow for a certain degree of linkage between loci (Falush *et al.* 2003) as well as other modifications.

TESS

Spatially explicit Bayesian clustering methods such as TESS (Chen *et al.* 2007; Durand *et al.* 2009b), BAPS5 (Corander *et al.* 2008) and GENELAND (Guillot *et al.* 2005) aim at identifying spatial population structure and spatially locating discontinuities in allele frequencies (e.g. Coulon *et al.* 2006). These methods can be used to detect the spatial population structure but also clines that are spatial trends in allele frequencies or genetic diversity resulting from either an adaptation across an environmental gradient or a secondary contact area between two previously isolated populations (Francois & Durand 2010).

In this dissertation, we have used TESS. It is a spatially explicit Bayesian algorithm which assumes that there are K_{max} populations that are at HWE. Geographical coordinates of the individuals are included in the prior distributions of the individual population assignment probabilities or admixture proportions. An individual spatial network is created based on sampling locations using statistical computations that are not described here (see Chen *et al.* 2007; Durand *et al.* 2009a; Durand *et al.* 2009b for further details). In the model without admixture, individuals that are spatially close in the network are given a higher probability to belong to the same population than more distant individuals. An interaction parameter controls the weight given to the spatial information, if it is null the model is similar to the model without admixture with uncorrelated allele frequencies of STRUCTURE. It should be noted that TESS does not allow modeling correlated allele frequencies. In the model with admixture, the fraction of an individual's genome that originated from each K is estimated. By incorporating spatial information, closer individuals should be more similar than distant ones. The interaction parameter controls the intensity of the spatial effect.

The most likely number of populations (K_{max}) is chosen using Deviance Information Criterion (DIC) which is similar to the ΔK method introduced earlier for STRUCTURE.

However, sometimes the effective number of populations (K) might be smaller than K_{max}. Thus, it is suggested to choose the value of K_{max} when it reaches a plateau (Durand *et al.* 2009a).

The parameters of interest are inferred in a Bayesian framework. Similarly to what we have seen for STRUCTURE, TESS produces barplots of assignment probabilities or admixture proportions. In addition, the main feature is the possibility to map the results (see Figure 2.5).

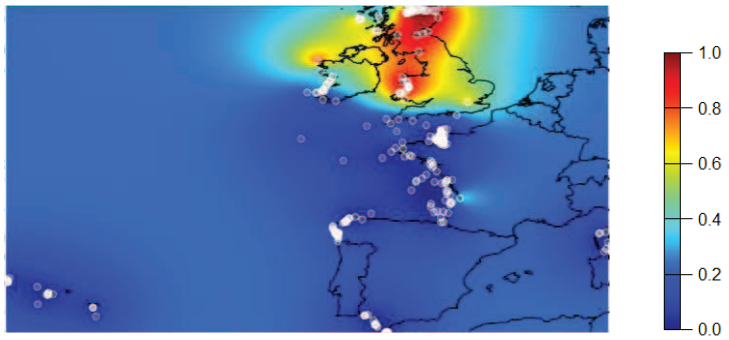
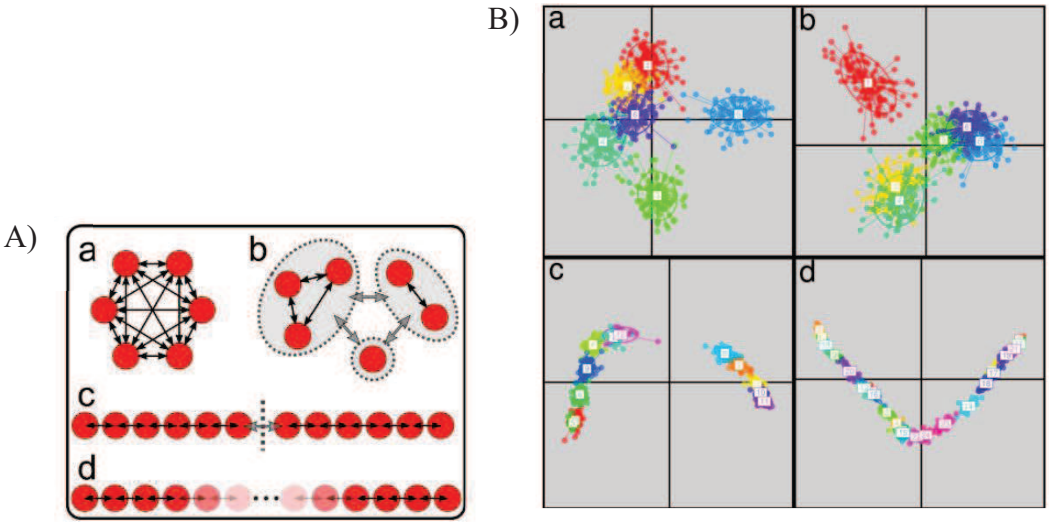


Figure 2.5. How to read TESS results? Each map produced by TESS is a map of the assignment probabilities (or admixture proportions) to one of the population (i.e. if the most likely number of population is four, four maps will be produced). Each individual is represented by a white point. The color scale represents the probability of each individual (or its genome) to be part of the population. The warmer the color, the higher the probability to belong to the population. Here, individuals sampled in Wales, Scotland and North-West Ireland have a high probability to belong to the same population.

Non-bayesian clustering methods

Both TESS and STRUCTURE rely on genetic model assumptions (e.g. Hardy-Weinberg and Linkage Equilibria) and are therefore based on an "idealized" population model. With large datasets, they may require long computational times, due to the nature of MCMC simulations, in particular for STRUCTURE. For example, the MCMC may need tens of thousands of steps to reach convergence. In addition, an initial portion of the MCMC should be discarded to avoid the influence of initial values on the posterior distributions. DAPC (Discriminant Analysis of Principal Components) is an alternative method that does

not rely on any genetic model assumptions (Jombart *et al.* 2010). It tries to cluster individuals based on genetic similarity, with genotypes being treated like a classical multivariate dataset. In DAPC, the number of clusters is first determined using a *K*-means method that aims at determining populations of individuals by minimizing within-population genetic variation. As in the Bayesian clustering methods, the *K*-means algorithm is ran with different numbers of putative populations. BIC (Bayesian Information Criterion) is used to determine the most likely number of populations. Then, the data are transformed using a Principal Component Analysis which summarizes the overall variability among individuals both among and within populations. This step ensures that the numbers of variables (i.e. alleles) are lower than the number of individuals and that the variables are not correlated. The Discriminant Analysis is applied on the Principal Components; it aims at partitioning genetic variation so that among-population variation is maximized while within-population variation is minimized. Individuals are assigned probabilistically to each population. DAPC has the advantage to have a fast computational time, even for large datasets. In addition, it has been shown to be as efficient as STRUCTURE (Jombart *et al.* 2010). DAPC also provides a visual representation of the structure between the populations, i.e. the scatterplots, which helps to understand the patterns of genetic structure (see Figure 2.6, Jombart *et al.* 2010).



Figures 2.6. Different migration models used to simulate data for DAPC analyses in Jombart (2010) for A)a) an island model, b) a hierarchical island model with the dotted lines indicating the archipelagos, c) a hierarchical stepping stone with the contact zone indicated by the dotted lines and d) a stepping stone. Red circles correspond to random mating populations and the arrows to gene flow with black arrows corresponding to a higher migration rate than grey ones. B) DAPC scatterplots of the simulated data for the four migration models (in the same order as in A, source: Jombart *et al.* 2010).

c) Coalescent theory and population demographic history analyses

Coalescent theory is the base of numerous methods or models that aim at reconstructing the past history of populations such as their size, growth rate, gene flow or their patterns and times of divergence using molecular markers. Here, I will explain the general theory and the specific method that was used in this dissertation to reconstruct the demographic history of bottlenose dolphins in the North-East Atlantic in Chapter 6.

Classical population genetics is a prospective approach which aims at predicting the future of allele frequencies in populations. In contrast, coalescent theory is a retrospective approach which aims at reconstructing the genealogy of a sample of genes going backwards in time to the Most Recent Common Ancestor (MRCA, Figures 2.7a to 2.7c, reviewed in

Nordborg 2001). It should be noted that in a coalescent framework, we work with genes, not individuals. In any population, the probability for two genes to coalesce follows an exponential probability distribution. As we get backwards in time, the number of genes will decrease and the time to the next coalescent event (represented by the branch length) will increase. As most mutations can be considered neutral, they can be added afterwards following a Poisson distribution with parameter the length of branches.

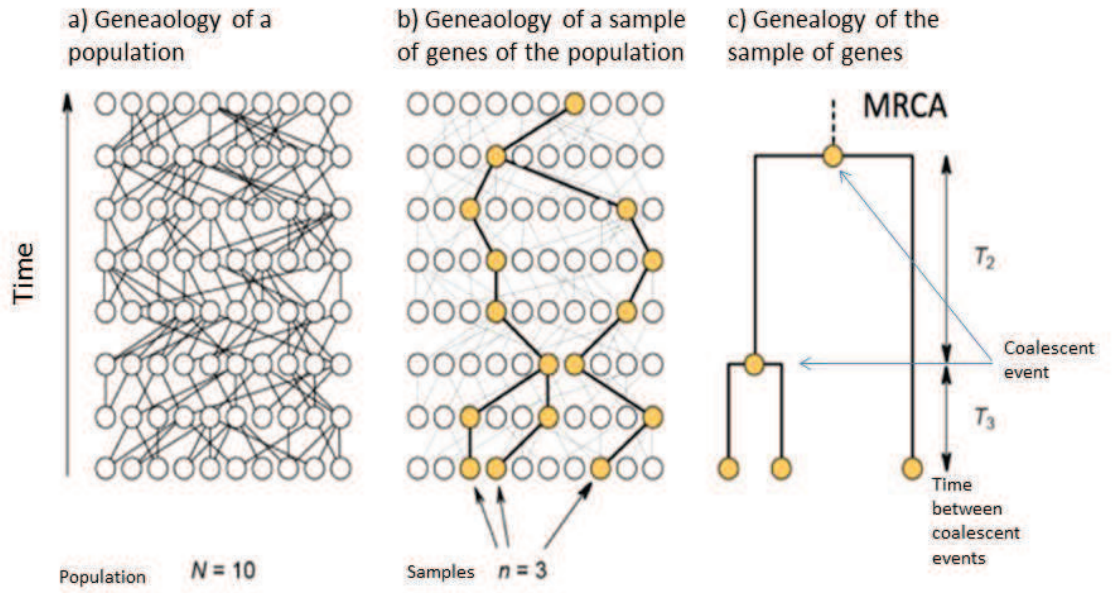


Figure 2.7. Principle of the coalescent theory. a) The complete genealogy of a population of 10 genes. b) Genealogy of a sample of genes (n=3), here highlighted in black, back to a single common ancestor. c) The genealogy of the sampled genes. It starts form *n* genes at present back to a single gene in the past, the Most Recent Common Ancestor (MRCA), through coalescent events at different times in the past (source: Leblois, 2010, "La théorie de la coalescence et ses applications", diapositives de cours, ENS Lyon).

For neutral markers, the gene genealogy is only based on the demography of the population. The topology of the coalescent tree (i.e. the branch lengths and times of coalescent events) can thus inform us about the demography of the population (Figure 2.8).

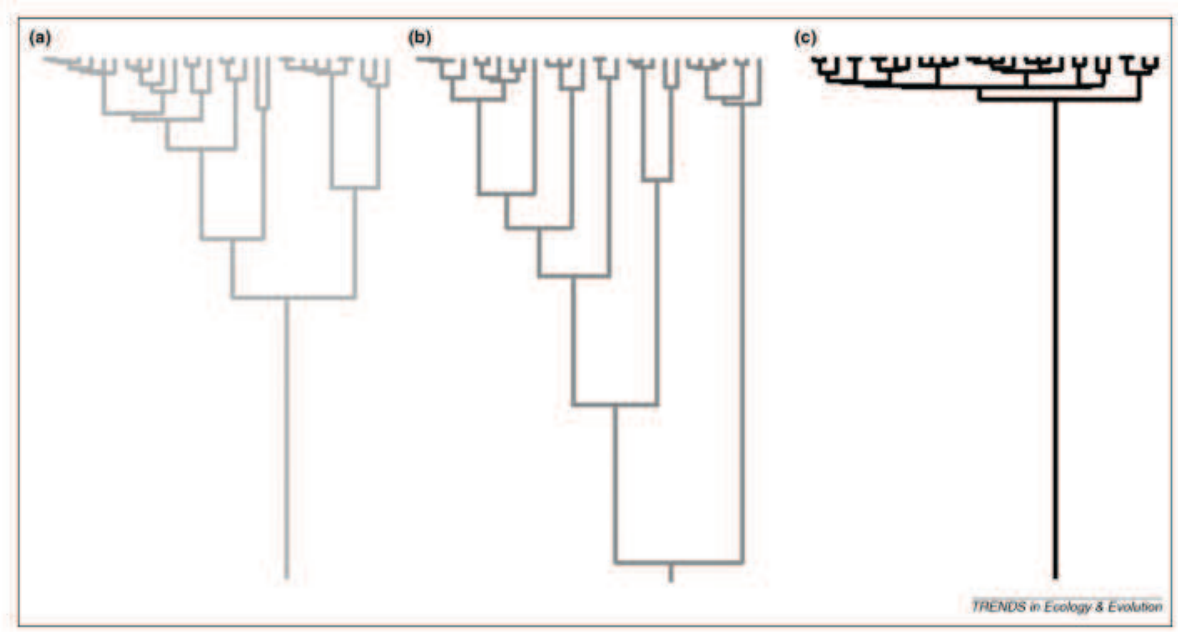


Figure 2.8. Genealogies sampled respectively from a) constant-size, b) shrinking and c) growing populations (source: Kuhner 2009).

The coalescent theory allows the probabilistic simulation of genetic variability expected under different demographic scenarios. Simulation is made easier as it is based on samples of genes instead of the whole population. However, the number of possible gene genealogies is infinite. Therefore, numerical approaches (that will not be detailed here) have been developed to explore the relatively more probable genealogies. These methods can be named "coalescent samplers" (reviewed in Kuhner 2009). To find the most likely genealogy (i.e. the probability that the data have evolved under this genealogy and mutation model), the sampler can implement either or both likelihood-based or Bayesian approaches using Markov Chain Monte Carlo (MCMC). However, the computation of the likelihood function is notoriously difficult, as the search space for parameters is infinite, which limits the possibilities of scenarios to test. Hence, mostly simple scenarios, which generally involve a low number of populations, can be tested. Although recent developments allow to include more populations (e.g. IMa2, Hey 2010), computation times are long (several months) and MCMC might never reach convergence as the parameter space is very large.

Another approach was also developed: "Approximate Bayesian Computation" (ABC) where the likelihood function calculation is replaced by simulations and summary statistics

which are used to measure the similarity of the observed and simulated datasets (reviewed in Bertorelle *et al.* 2010; Csilléry *et al.* 2010). In this dissertation, we have used the computations implemented in the software package DIYABC (Cornuet *et al.* 2008; Cornuet *et al.* 2010; Cornuet *et al.* 2014). A large number of demographic scenarios can be tested, which can combine admixture and divergence between populations and changes in effective population sizes and can include a large number of populations. For instance, patterns and times of divergence among populations, colonization events, or changes in effective population sizes can be investigated (e.g. Verdu *et al.* 2009; Estoup & Guillemaud 2010; Fontaine *et al.* 2012). One drawback of the DIYABC program is that it cannot explicitly include migration. In DIYABC, similarly as in other ABC approaches, the different steps are as follow (and are summarized in Figure 2.9 adapted from Excoffier *et al.* 2005; Cornuet *et al.* 2008):

1) Simulation step:

Simulated datasets are generated under demographic scenarios and mutation models with parameter values drawn from prior distributions. Prior distributions include *a priori* knowledge on the population of interest (e.g. effective population sizes) and the markers (e.g. microsatellites and mitochondrial DNA mutation rates for mammals). Simulations are based on coalescent theory. Summary statistics are selected and computed on the observed and simulated datasets. The summary statistics correspond to quantities used to characterize genetic diversity within and among populations (e.g. the number of alleles or F_{ST}). The choice of the statistics depends on the demographic history questions to investigate.

In DIYABC, the ability of the combinations of scenarios and priors to produce simulated summary statistics that are close to the observed summary statistics can be checked using a Principal Component Analysis or by statistically comparing each summary statistic of the observed data to the distribution of the simulated summary statistics. This step can help to determine if some parameters of the model or the priors have not been well defined.

2) Selection step:

Euclidian distances between simulated and observed summary statistics are computed and the simulated datasets which are closer to the observed dataset are selected (e.g. 1%) while the others are rejected.

3) Estimation step and scenarios comparison:

The posterior probabilities of each scenario can be estimated and compared between scenarios, using the simulated datasets which are the closest to the observed dataset, by two different methods: by calculating how much time each scenario is found (the direct approach) or by applying a logistic regression (which should be preferred, Beaumont *et al.* 2002). In the regression, the posterior probability of scenarios is the dependent variable and the predictors are the distances between observed and simulated summary statistics.

The posterior distributions of the parameters for each scenario are estimated by applying a local linear regression to the simulated datasets which are the closest to the observed one. In the regression, the parameter is the dependent variable and the predictors are the distances between observed and simulated summary statistics.

4) Confidence in the scenario choice and in the parameter estimates

For each scenario, a few hundred datasets are simulated using parameters values drawn from the prior distribution specified in the first step. Posterior probabilities are computed and used to estimate the Type-I and Type-II error rates in choosing each scenario. For instance, Type-I error rate for scenario A is estimated as the proportion of simulated datasets generated under scenario A that supports other scenarios. Type-II error rate for scenario A is estimated as the proportion of datasets simulated under all the other scenarios that supports scenario A.

5) Model-checking

The "goodness-of-fit" of a scenario according to the observed dataset, that is how well a scenario can reproduce the observed dataset, can be computed. It measures the consistency between a scenario and its parameter posterior distributions (i.e. "the posterior predictive distributions") and the observed dataset using summary statistics. Summary statistics should also include statistics that have not been included previously in the inference step; otherwise the quality of the fit may be overestimated. In practice, data are simulated under each scenario using parameter values drawn from parameter posterior distributions. DIYABC allow testing visually, through a Principal Component Analysis, if the observed data are in the range of the values generated using the posterior predictive distributions. The probability that the simulated data do not encompass the observed data could be estimated for each summary statistics.

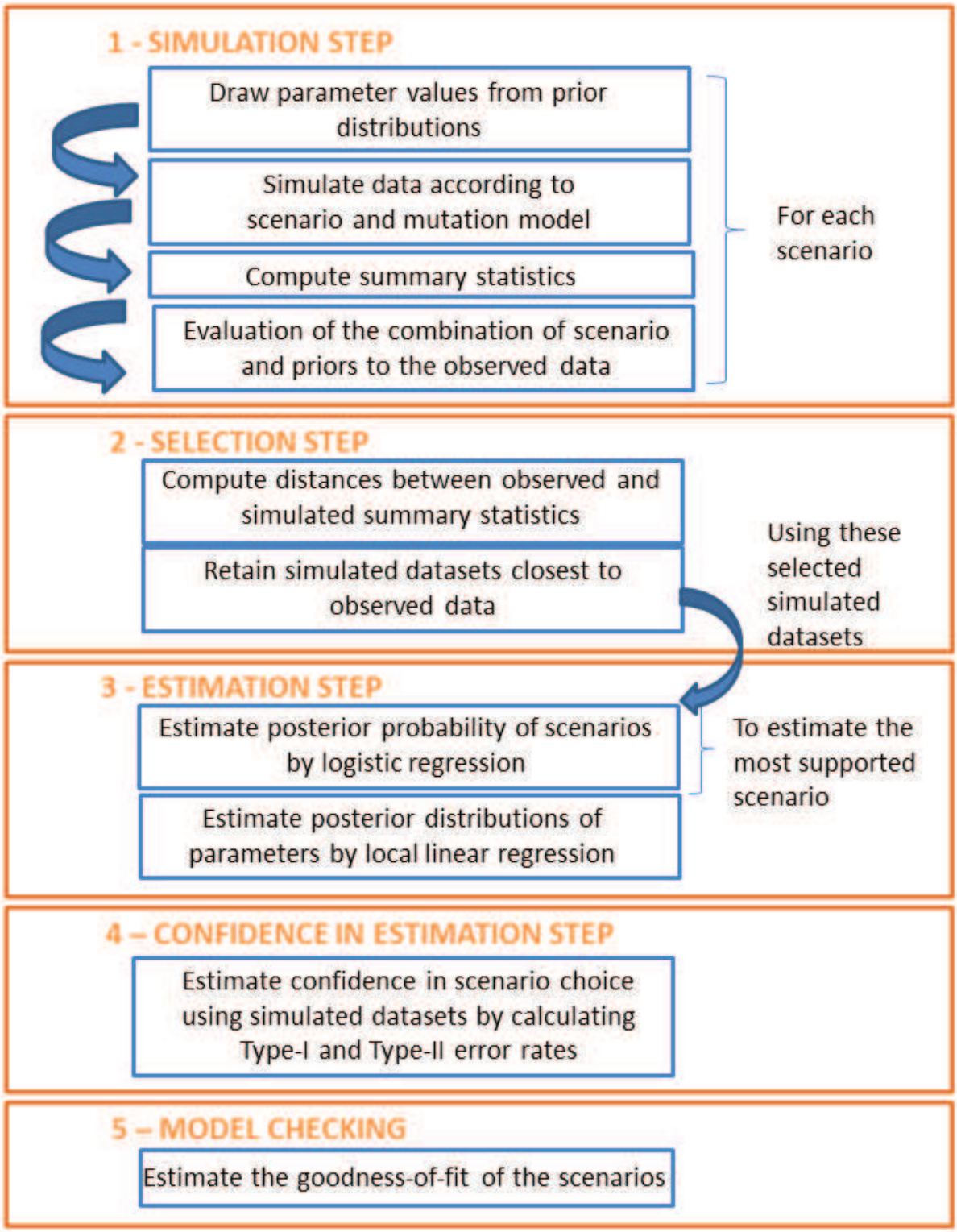


Figure 2.9. The different steps of an Approximate Bayesian Computation Analysis in DIYABC (source: adapted from Excoffier *et al.* 2005; Cornuet *et al.* 2008; Cornuet *et al.* 2010).

CHAPTER

3

SOCIAL STRUCTURE AND ABUNDANCE OF COASTAL BOTTLENOSE DOLPHINS IN THE NORMANO-BRETON GULF, ENGLISH CHANNEL

1) Introduction

The estimation of spatio-temporal variations of demographic parameters in top predator populations is critical to assess their health, the potential impact of anthropogenic activities and to take appropriate management measures (Frederiksen *et al.* 2004; Votier *et al.* 2005; Bejder *et al.* 2006). In addition, for social species, studying the social structure and differences in habitat use between social clusters, i.e. sets of individuals so that the majority of social associations occurs within rather than between social clusters, is also important to ensure their conservation (Sutherland 1998; Whitehead *et al.* 2004). For example, according to their spatial distribution or diet specializations, distinct social clusters may respond differently to human activities or environmental changes (Chilvers & Corkeron 2001; McComb *et al.* 2001; Whitehead & Rendell 2004; Whitehead *et al.* 2004; Ansmann *et al.* 2012a). Studying social structure can also shed light on the forces that are driving population processes. Sociality develops as a trade-off between the selective forces conferring benefits to group living (such as cooperation, protection from predators, transfer of information) and the costs incurred (e.g. increased competition, parasite load, see review in Krause & Ruxton 2002). Social groups are likely to be maintained when the fitness gains of sociality outweigh the costs (Alexander 1974). Ecological factors, in particular variations in local resources, can affect the size and persistence of social groups (Wrangham 1980; Rubenstein & Wrangham 1986; Lusseau *et al.* 2004; Ramos-Fernandez *et al.* 2006). For instance, in fission-fission societies, associations between individuals are highly dynamic and temporary, lasting from several hours to a few days, and may be adjusted in response to fluctuations in resource availability. Individuals tend therefore to associate when fitness benefits of social grouping are high (Connor *et al.* 2000; Wittemyer *et al.* 2005; Smith *et al.* 2008). Individuals can also share long-lasting and stable relationships and the proportion of long-term associations might be constrained by ecological conditions (as suggested in Lusseau *et al.* 2003; Augusto *et al.* 2011).

Bottlenose dolphins (*Tursiops* sp.), which are found from temperate to tropical waters, live in fission-fusion societies (Connor *et al.* 2000). They associate in small groups whose composition quickly changes (several times per day). Associations tend to be determined by gender and age (Connor *et al.* 2000). However, in these dynamic societies, besides mother

and calf associations that typically last for at least 3 years (Wells *et al.* 1987), individuals can also share strong relationships such as those among adult males (Connor *et al.* 1992). Social structure varies across communities (i.e. groups of individuals of the same species that co-occur in space and time and have an opportunity to interact with each other) and seems to be shaped by ecological factors such as prey resources or oceanographic conditions and intrinsic factors, in particular, shared knowledge and behavioral strategies (Lusseau *et al.* 2003; Daura-Jorge *et al.* 2012; Mann *et al.* 2012). Great variations in distribution and size of communities have also been reported worldwide, with communities exhibiting patterns of residency ranging from highly resident (Wilson *et al.* 1999) to migratory (i.e. showing seasonal site fidelity, Barco *et al.* 1999) or transient (i.e. showing no site fidelity, Defran & Weller 1999). Abundance vary also from very small communities of tens (Liret 2001) to very large communities of thousands of individuals (Read *et al.* 2003).

Tursiops truncatus is the only bottlenose dolphin species occurring within European coastal waters (Hammond *et al.* 2012). Bottlenose dolphins are protected under European Union's Habitats Directive (92/43/22C) where they are listed in Annex II as a species whose conservation requires the designation of Special Areas of Conservation and in Annex IV as in need of strict protection. They are observed from Iceland to the Strait of Gibraltar as well as in the Mediterranean Sea and the Black Sea (Hammond *et al.* 2012). The species occurs both in pelagic and coastal areas (Hammond *et al.* 2013) where it can be impacted by increasing human activities (e.g. Pirotta *et al.* 2013). Three resident communities are found in French coastal waters of the Atlantic and the English Channel: two small communities (tens of individuals) in the Iroise Sea (one off Sein Island and the other one off Molene Island, Liret 2001), and a community in Normandy coastal waters (the Normano-Breton gulf, also known as the gulf of Saint-Malo and named the gulf hereafter, Figure 3.1 in the material and methods section).

In this chapter, we focused on bottlenose dolphins of the Normano-Breton gulf, which remain poorly known although they are the most commonly encountered cetacean species in the area (GECC, personal communication). They are genetically isolated from the neighbouring communities in the United-Kingdom and Ireland (see Chapter 5). Furthermore,

they inhabit an area of special interest. First, a marine park is under creation. A marine park is a marine protected area (IUCN category V "protected seascape"), which promotes sustainable development of human activities together with biodiversity monitoring and protection. Second, human activities are increasing in the area, several large-scale marine renewable energy constructions are planned in the upcoming years. The construction of wind farms in the North and Baltic Seas has impacted the distribution of harbour porpoise (*Phocoena phocoena*), and their displacement was linked to the loud sound produced by pile-driving events (Carstensen *et al.* 2006; Tougaard *et al.* 2009). In this context, it is important to carry out studies on the bottlenose dolphin community in the gulf several years prior to the beginning of the building of these extended wind farms to gather benchmark data on the community before any potential impacts are manifested.

The goal of this study was therefore to provide baseline knowledge on social structure and abundance of this bottlenose dolphin community both for its monitoring and management and for research questions on the drivers of social structure. Despite being extensively studied, social structure research projects in different habitats across the broad range of bottlenose dolphins can contribute towards a better understanding of the forces shaping sociality in the species. First, grouping patterns were examined and the social structure of the community was investigated using association and lagged association rate analyses. It is essential to define whether there were any completely discrete social clusters before estimating abundance to determine if it is relevant to estimate it for the whole community or for the different social clusters. The second objective was to estimate the size of the community frequenting the gulf using mark-recapture models.

2) Material and methods

a) Surveys and photo-identification

From 2006 to 2010, year-round boat surveys were performed in the Normano-Breton gulf, Normandy, France by the GECC (Groupe d'Etude des Cétacés du Cotentin) whenever sea state was favourable (i.e. sea state < 3 Beaufort). The aim of these surveys was to photo-identify bottlenose dolphins using the gulf waters. From 2007 to 2010, Global Positioning System tracks of the surveys were recorded together with observation effort and dolphin group encounter data. The search effort (i.e. the gps track records of the boat when dolphins were not followed) was represented in R version 3.0.0 (R Development Core Team 2013) using marmap package (Pante & Simon-Bouhet 2013, Figure 3.1). The first contact point was reported on the map for each group encounter. The study area was not homogeneously surveyed during the study period. At first, surveys were initiated within the southern part of the gulf. Then the survey area was extended to the central part of the gulf from 2007 onwards. A single survey week was conducted in the northern part in 2007 and the surveys were extended to the northern area in 2008. In 2008 and 2009 the whole gulf was surveyed, but the spatio-temporal coverage was not homogenous. In 2010, the whole area was surveyed regularly.

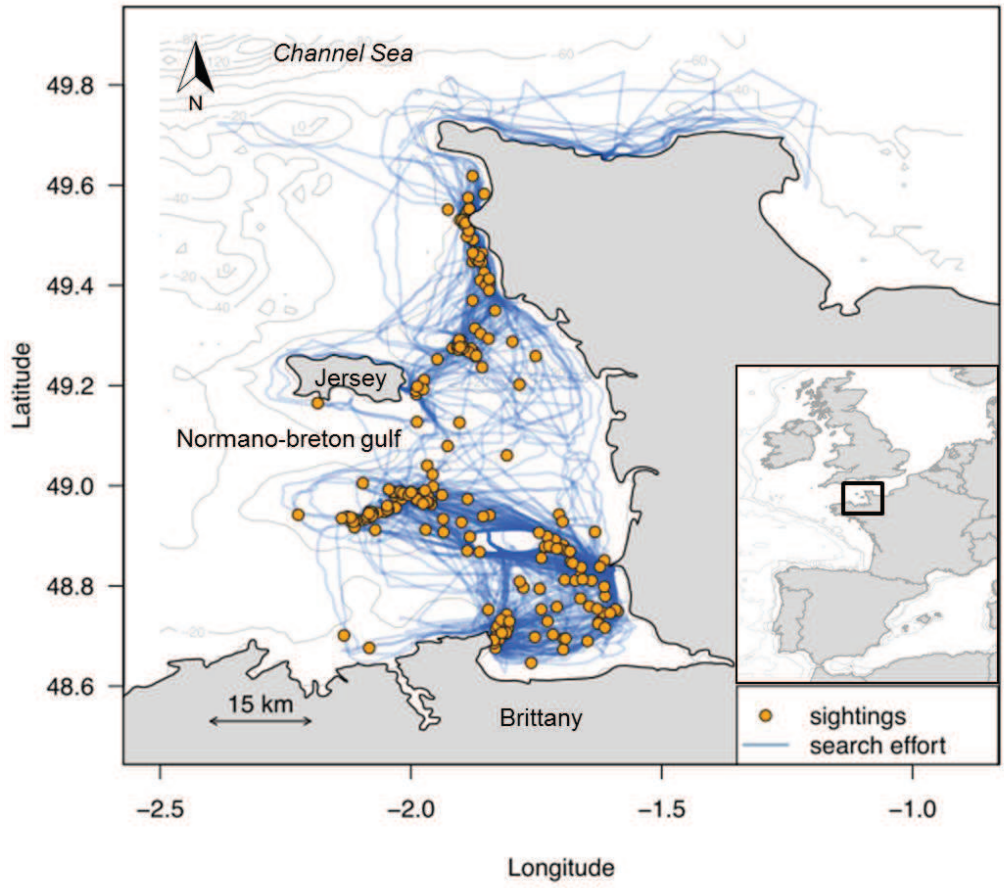


Figure 3.1. Location of the study area, distribution of survey effort (i.e. boat gps trackings when searching for dolphins) and location of sightings of bottlenose dolphins from 2007 to 2010.

During surveys, dorsal fins and upper backs of encountered individuals were photographed. Individuals were identified using natural marks: scars, nicks and scratches on their dorsal fins (Würsig & Würsig 1977; Würsig & Jefferson 1990). A catalogue was created and used to re-identify individuals. When available both sides of the dorsal fin were included in the catalogue. A marking level (M), according to the number and size of the nicks, was attributed to each individual (Figure 3.2). It varied from M1 for individuals with a smooth dorsal fin with scratches to M4 for strongly marked individuals (numerous and big nicks). Individuals with a smooth dorsal fin and without any or few slight scratches were considered as unmarked and were not entered in the catalogue. Quality of the fin photographs was assessed using three grades (excellent, good and poor) which are dependent on several criteria in particular the focus, angle of the animal, presence of water splashes, proportion of the fin

out of the water and the distance to the photographer. Only good and excellent fin photographs, taken on either side of the fin, were used for photo-identification. If there was any doubt in the identification, dolphins were not identified. As several people worked on photo-identification data, in order to minimize errors, I double-checked all the identifications over the whole period. We calculated the cumulative number of identified dolphins for each year.

Figure 3.2. Bottlenose dolphin marking levels.

b) Social structure

Social structure was investigated using data collected between 2006 and 2010. Individuals were considered associated if they were observed in the same group. A group was defined as all dolphins within an area of 100 m radius involved in similar behavioral activities (Wells *et al.* 1987). A sighting refers to the encounter of a group or the encounter of an individual within a group. Group size was estimated visually by at least two experienced observers. Photo-identification work started when the first dolphin was spotted, it lasted as long as the dolphins were in the sight of the observers and ended usually when the surveyors decided that they had enough photographs of the animals or when dolphins showed boat avoidance behavior signs. Attempts were made to photograph all the animals, whatever their levels of markings. The statistical analyses conducted here are robust to the non-identification of some members of a sampling unit (see below). Thus, we did not exclude any group from the analyses (e.g. based on the proportion of individuals photographed). Social structure analyses were run using SOCPROG 2.4 (Whitehead 2009a) implemented in Matlab 7.6.0. (Mathworks Inc., Natick, MA, U.S.A.). A daily sampling period was used to avoid

demographic effects (such as death, emigration or immigration) and we excluded the individuals that were identified only on four or fewer occasions to minimize the bias due to these poorly sighted individuals. However, the choice of an appropriate cut-off is not straightforward and various values were used in the literature (e.g. Lusseau *et al.* 2006; de Stephanis *et al.* 2008c; Wiszniewski *et al.* 2009; Ansmann *et al.* 2012a). Whitehead recommends a minimum of five identifications (Whitehead 2008a). We performed analyses on individuals identified in at least five to twelve sampling periods. Since results with six to twelve identifications were similar to those with five identifications but included far less individuals and were therefore less representative of the field data, we only present here the results that include animals identified in at least five sampling periods. Individuals with a smooth dorsal fin (M1) were excluded from analyses as their scratches could change quickly. Moreover, as their scratches are only visible on one side, it is difficult to identify them on both sides and this could lead to misidentifications. Therefore, only marked adults and sub-adults were considered in these analyses as newborns and young animals are generally difficult to identify due to their low level of marking.

The Half-Weight Index (HWI) was used to quantify the strength of associations between pairs of individuals. This index minimizes bias if not all the associates are identified (Cairns & Schwager 1987). Since the Half-Weight Index is commonly used in bottlenose dolphin social structure studies this makes comparisons between studies easier.

The index is described by:

$$HWI = \frac{X}{X + 1/2(Ya + Yb)}$$

where X is the number of groups where dolphins *a* and *b* were seen together, *Ya* is the number of groups where dolphin *a* was observed without dolphin *b*, and *Yb* is the number of groups where dolphin *b* was observed without dolphin *a*. It ranges between 0 (*a* and *b* never seen together) to 1 (*a* and *b* always observed together). Standard deviation (SD) and coefficient of variation (CV) of the HWI were also calculated.

A Monte Carlo permutation test was conducted to determine whether observed association patterns were significantly different from random association patterns using the recommendations of Bejder *et al.* (1998) with modifications included in Whitehead (1999, 2008a, 2009a). The matrix of observed association indices was permuted within sampling periods until *P* stabilized at 10,000 permutations with 100 flips. The test was then run three additional times to ensure *P* stability. A higher standard deviation (SD) of the observed association indices in comparison to the SD of permuted data indices shows that long-term preferred and/or avoided associates were present in the community (Whitehead 1999, 2008a).

Reliability of the social structure representation was assessed using the Pearson correlation coefficient (*r*) and the social differentiation (*S*) (Whitehead 2008a, b). We estimated the accuracy of the social structure representation by correlating estimated association indices with their true value using the maximum likelihood estimator *(r* = 0 for an inaccurate representation; $r = 1$ for an excellent representation). The social differentiation, which is the coefficient of variation of association indices estimated using maximum likelihood, gives the variability of association indices in the community. A value of *S* close to 0 indicates that association indices are homogenous in the community and a value of *S* equal or greater to one that they are highly variable. Fewer data are needed to accurately reconstruct social structure when the social differentiation is moderate or high (i.e. superior to 0.5, Whitehead 2008a, b). Standard Errors (SE) were calculated for *r* and *S* from bootstrap with 1,000 replications.

The social structure of the community was examined using a hierarchical cluster analysis with the average linkage method on the HWI matrices. The average linkage method is considered as the most accurate method to display social structure in clusters because outlier distances have less impact on the results than with single or complete linkage methods (Milligan & Cooper 1987; Whitehead & Dufault 1999). It is therefore the most commonly used method in social structure analyses (e.g. Lusseau *et al.* 2003; Wiszniewski *et al.* 2009; Augusto *et al.* 2011). We assumed that a cluster with a cophenetic correlation coefficient (i.e. the correlation between observed dyadic association indices and the indices represented in the dendrogram) higher than 0.8 indicated a reliable separation between clusters (Whitehead

2008a). The most parsimonious cut-off in the cluster was defined using the division that maximizes the modularity coefficient *Q* (Newman 2004; Lusseau 2007; Whitehead 2008a) which is defined as the difference between the proportion of the total association measured within clusters and the expected proportion if pairwise association indices were randomly distributed. Therefore, this method divides the individuals into clusters where association indices are higher among members of the same cluster than expected by chance. The analysis takes into account differences of gregariousness (i.e. mean number of associates of an individual) among individuals. A modularity coefficient of 0 shows a random group structure. A value equal to or greater than 0.3 indicates a good division between clusters (Newman 2004). We ran a Mantel test to test if association indices were significantly higher inside each of the social clusters than between them in R. 3.0.0 (R Core Team 2013) using ade4 package (Chessel *et al.* 2004; Dray & Dufour 2007; Dray *et al.* 2007). We compared mean association indices of individuals within and between clusters.

In order to visualize if social clusters were spatially distributed, the median latitude/longitude of the sighting positions of each individual was calculated. As we included individuals with few identifications (minimum identifications set to five), median position was chosen since it is more robust to outlier positions than the mean. We also calculated Median Absolute Deviation (MAD) to take in account of the variability in the sighting positions (Venables & Ripley 2002). Median position and MAD for each individual were then represented on a map that also indicated its social cluster to examine whether ranging differences can account for social structuring of the community.

To determine the temporal stability of associations among individuals, we calculated variations in standardized lagged association rates (SLAR, Whitehead 1995; Whitehead 2008a). SLAR is the probability, given that *a* and *b* are associated at time 0, that *b* will be randomly chosen as associate of *a* after a specified time lag. The probability was averaged over all individuals. The SLAR is robust to the non-identification of all the associates. This average standardized association rate was estimated by $g(\tau)$ as defined by Whitehead (1995) and plotted in relation to time lag (in days). All individuals (even rarely observed individuals) were considered for this analysis as poorly observed animals will have little impact on the

SLAR estimation (Whitehead 2008a). SLAR was compared to the standardized null association rate (NAR), which represents the SLAR when there are no preferred associations, to determine whether the patterns of associations were non-random (Whitehead 1995, 2008a). Then, four exponential decay models of temporal stability were fitted to the dataset (Whitehead 1995, 2008a). These models take into consideration two types of associations: constant companionships (that last until death) and casual acquaintances (associations lasting from a few days to a few years). Each model was composed of a combination of these two types of associations (Table 3.1 in the result section). The rapid dissociations (associations lasting a few hours) were not incorporated directly in the models as they were confounded with gregariousness. Therefore each of the four models may or may not have included rapid disassociations (Whitehead 2008a). The model that best described the temporal dynamics of the social structure was selected by the Quasi-Akaike Information Criterion (QAIC, Whitehead 2007). The precision of the parameters was estimated using jackknife (Efron & Stein 1981; Whitehead 1995, 2008a).

c) Abundance

Mark-recapture models were applied to photo-identification data to estimate the size of the community. For this analysis, only individuals of marking levels M3 and M4, unambiguously identifiable on both sides, were included to minimize identification errors. We estimated the total community size using survey data collected from July to September 2010 over the whole study area of the Normano-Breton gulf. For the 2006-2009 period, no particular effort was made to survey the whole gulf as regularly as in 2010 making it impossible to reliably estimate abundance without spatial bias.

During the 2010 summer season, seven capture occasions were conducted from July 10th to September 18th 2010. Each capture occasion was composed by two whole day surveys (one survey in the northern part and another in the southern and central parts of the gulf) in order to cover the whole area. Efforts were made to minimize the time between each of the two surveys inside a capture occasion but it varied with weather conditions, they were usually carried out one to two days apart. In two occasions, surveys were carried out

simultaneously on the same day, using two boats. Summer was chosen for abundance estimation as weather permits more regular surveys than during other seasons.

Abundance of well-marked individuals (*N*) was estimated in MARK (White & Burnham 1999). Among the standard sequential mark-recapture models for closed populations (Otis *et al.* 1978), we compared models M_o, M_h, M_t, and M_{th}. Capture probabilities could vary between individuals (*h*) and with time (*t*) because of a variety of factors such as avoidance or attraction to the boat (Hammond 1986), individual differences in home ranges, variations of survey effort and different photographers. We did not test models that assume a behavioral response to capture (M_b, M_{tb}, M_{bh}, M_{tbh}) as photo-identification is a non-invasive method. It is therefore common to exclude models assuming a behavioral response in photo-identification studies (e.g. Wilson *et al.* 1999; Daura-Jorge *et al.* 2013). Heterogeneity among individuals (*h*) was modeled using two mixtures. Standard models (M_o and M_t) were built from finite mixture models (M_h and M_{th}), setting the mixture parameters to 1.

The following assumptions were made for the tested models:

1) The population was closed demographically (no death or birth) and geographically (no emigration or immigration) during the time period considered.

2) All marked individuals were correctly identified and recorded on each capture occasion.

3) Marks were not lost and marked individuals were not preferentially photographed.

The sampling period was short (two months), so there was a strong probability that the demographic closure assumption was respected. Dolphins are indeed long-lived animals with a low reproduction rate. Emigration and immigration could not be totally excluded but could be considered minimal due to the short sampling period. The closure assumption was confirmed using Close Test (Otis *et al.* 1978; Stanley & Burnham 1999). Assumptions 2 and 3 were fulfilled as only well-marked and easily-identified individuals with good to excellent fin photographs were included in analyses. Mark changes could occur, however as the

sampling period was short and the surveys were regular, we assumed that any mark changes could be detected.

Models were compared using AIC_c (which is adjusted to small sample size, Hurvich & Tsai 1989; Burnham & Anderson 2002, Table 3.2). We calculated AIC_c weights, which measure the support of a given model relative to the others. Based on the AIC_c weights, we estimated the average abundance across all models (Burnham & Anderson 2002). Log-normal confidence intervals were calculated following the formulas described in Lukacs (2013).

Abundance estimation (*N*) run in Mark was only based on well-marked individuals. Therefore, the mean proportion of well-marked individuals (M3 and M4) on the total number of fins (M1 to M4 and unmarked fins) was estimated. This was performed for each photo showing at least two dorsal fins. The mean proportion was calculated as follows:

$$\hat{\theta} = \frac{1}{n}\sum_{i=1}^{n} \frac{m_i}{t_i}$$

where n is the number of photos in the dataset, m_i is the number of heavily marked individual fins on photo i, t_i is the total number of individual fins on photo i. To estimate the whole community size (N'), N was adjusted with the calculated proportion: $N' = \frac{N}{\hat{\theta}}$. Confidence intervals were corrected following Whitehead *et al.* (1997).

3) Results

a) Survey effort and photo-identification

Between 2006 and 2010, 201 bottlenose dolphin groups were recorded on 134 field days. Photos were taken during 199 group encounters. A mean of 171 photos (SD = 216) per group were of sufficient quality to allow identification of at least one individual. A total of 336 marked individuals (M2, M3 and M4) and 361 M1 individuals were identified. Mean visually estimated encounter group size in the field was 26 (SD = 18, range: 1 to 100). 56% of visually estimated individuals in the groups were of marking levels M2 to M4 and were photo-identified. Mean identified M2, M3 and M4 individuals per group was 14 (SD = 13, range: 1 to 87). Attempts were made to photograph all individuals, it is however difficult to disentangle the proportion of missed individuals from the proportion of M1 (only scratches) and unmarked individuals. Among the 336 marked individuals, 32% were seen only during one year, 18% during two years and 50% during three or more years. The discovery curve of new well-marked (M3 and M4) individuals sharply increased between 2006 and 2007 (Figure 3.3). This increase corresponded to the expansion of the study area. It then tended to stabilize, indicating that we had identified most of these individuals and that immigration could be considered as low. However, when we included slightly marked (M2) individuals, the curve was still increasing in the recent years. These new individuals could either be previously smooth dorsal fin individuals (M1 or unmarked) that were already in the gulf or immigrants.

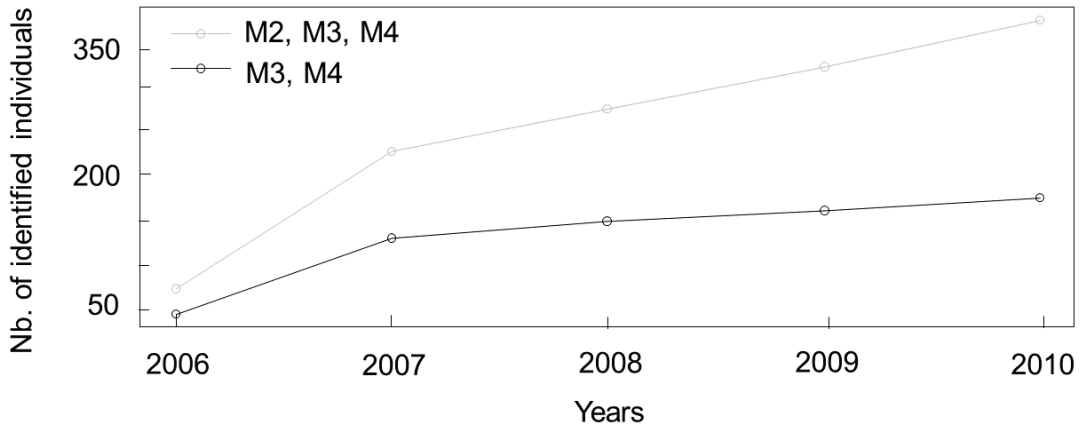


Figure 3.3. Cumulative number of identified individuals from 2006 to 2010 according to their marking levels (M2, M3 and M4).

b) Social structure

Eight group encounters were removed from the analyses as no marked individuals (M2, M3 or M4) or no individual identified in at least five sampling periods were included. Therefore 191 group encounters were used in the social structure analyses. A total of 206 marked dolphins (M2, M3 and M4) were identified in at least five sampling periods. They represented 88.92% (SD = 15.32) of all the marked dolphins identified in each group. We excluded from the analyses 130 dolphins that did not meet the minimum of five identifications criteria. The mean number of observations of all marked dolphins (M2, M3 or M4) was 6.41 (SD = 5.34). When considering individuals identified at least five times, the mean number of observations of an individual was 10.12 (SD = 5.42) and the maximum was 29 observations.

The mean Half-Weight Index (HWI) was 0.097 (SD = 0.136, CV = 1.396). This standard deviation was higher than the standard deviation obtained from permuted data (SD = 0.132, $P<0.001$), suggesting that individuals did not associate randomly and that there were long-term preferred or avoided companions in the community.

The correlation coefficient r between the true association indices and their estimates was 0.68 (SE = 0.04) indicating that the estimated association coefficients adequately represented social structure. Social differentiation was $S = 0.95$ (SE = 0.03), which indicated that relationship patterns were highly variable. S^2 * H (H: mean number of associations per individual) = 0.95^2 * 211 = 190, which is well above 5, indicating an excellent ability to reject the null hypothesis of no preferred/avoided associations (Whitehead 2008b). Therefore, our analyses had good power to detect the social system (Whitehead 2008a, b).

The cluster cophenetic correlation coefficient was 0.747, which is close to the 0.8 threshold indicating an effective social structure representation (Figure 3.4, Whitehead 2008a). Maximum modularity ($Q = 0.320$) at HWI = 0.085 provided a reliable separation in three different clusters (Figure 3.4, Newman 2004). One individual (represented by a black line in the Figure 3.4) was not assigned to any of the three clusters. However, this individual was mostly seen with individuals of the cluster “North” (83% of its identifications).

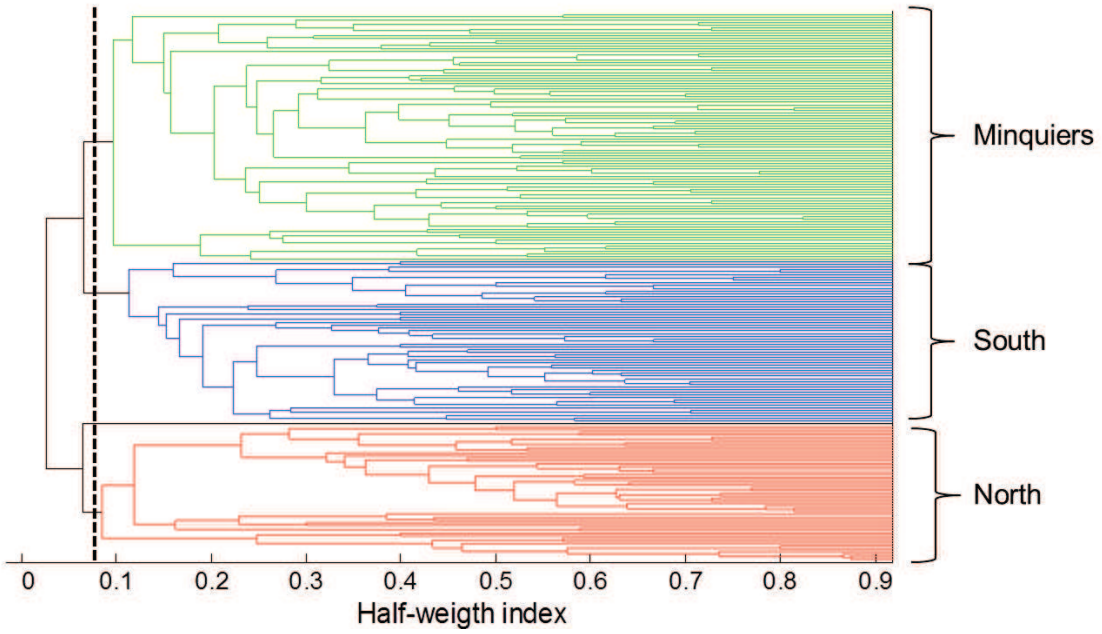


Figure 3.4. Hierarchical cluster analysis with the average linkage method of the Half-Weight Index matrices. Cluster division was obtained using maximum modularity controlling for gregariousness (modularity value was 0.320 and was maximized at HWI = 0.085 as indicated by the dashed line). Cluster Cophenetic Correlation Coefficient was 0.747.

Mantel test confirmed that there were significantly more associations among individuals of the same clusters than among individuals of different clusters (r = 0.55, P<0.001). Mean HWI among individuals of the same clusters was about twice of the HWI averaged on all individuals. Moreover, the mean association index between individuals of clusters "North" and "South" was very low (HWI = 0.007, SD = 0.029, Figure 3.5). The dendrogram (Figure 3.4) also showed that there were strong associations (equal or above 0.5) among a few individuals (1.93% of the total possible associations).

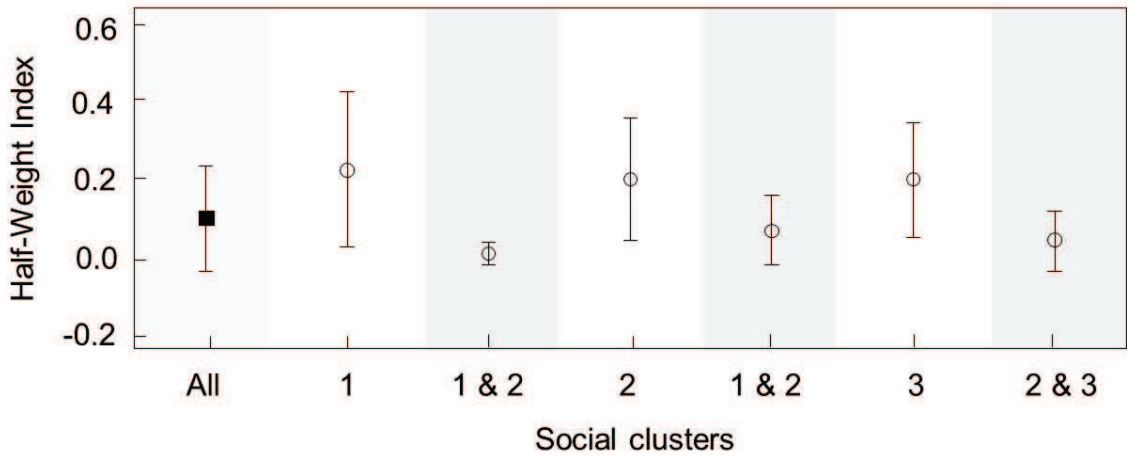


Figure 3.5. Mean Half-Weight Index calculated between all the individuals (ALL, light grey shading and filled square), between individuals of the same social cluster (no shading) and between individuals of two distinct social clusters (grey shading). Error bars indicate Standard Deviation.

The map showing the median geographical position of each dolphin's sightings as a function of their social cluster indicated that the clusters showed a degree of spatial segregation (Figure 3.6). Dolphins from cluster "North" were mainly observed in the northern part of the gulf, dolphins from cluster "Minquiers" in the center part, and individuals from cluster "South" were mainly observed in the southern part of the gulf. However, error bars (median absolute deviation) showed spatial overlaps between the localisations of individuals of different clusters.

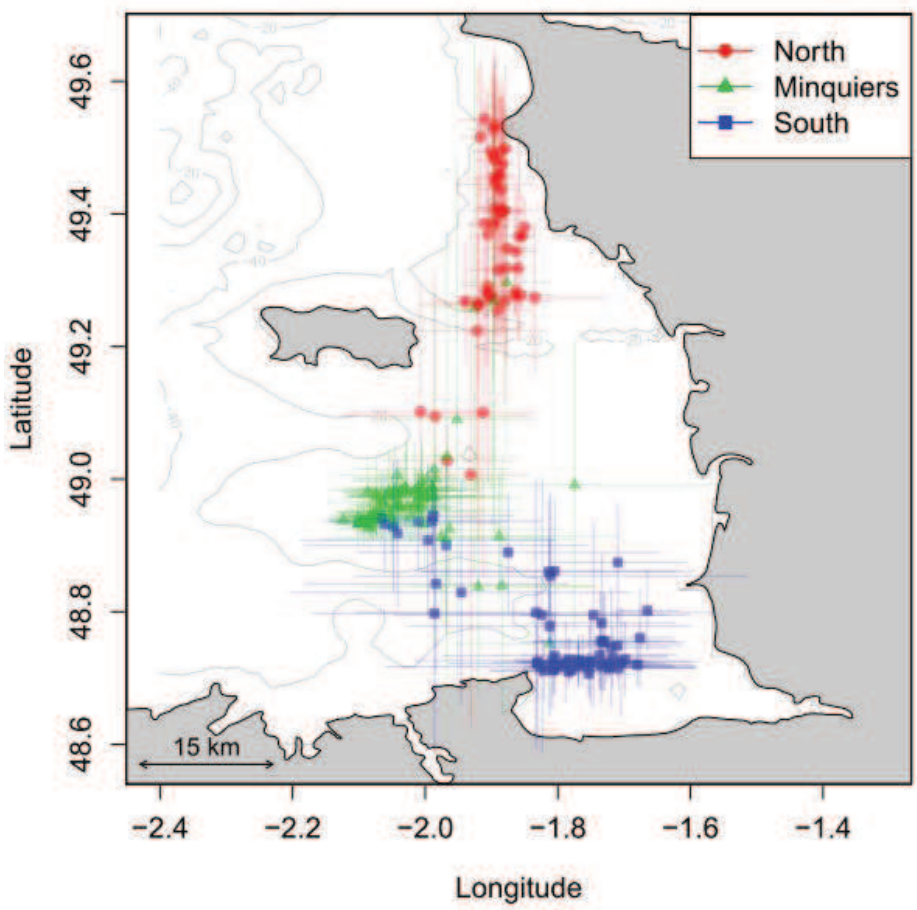


Figure 3.6. Median latitude/longitude of all the sighting positions for each individual (points) and standard error of median absolute deviation (arrows). Color and symbol codes indicate the social cluster of each individual.

The Standardized Lag Association Rate (SLAR) was different from the Null Association Rate (NAR) showing non-random temporal association patterns (Figure 3.7). The SLAR curve and error bars indicated high variability in the association durations. The curve dropped drastically after a few days and in spite of a high variability, continued to decrease until 100-200 days. The curve stayed slightly above the NAR indicating the existence of a small proportion of long-term companions. The model that best described the temporal stability of associations included casual acquaintances and constant companions (Table 3.1). The model indicated that the duration of the casual acquaintances was in the order of 80.56 (SE = 77.35) days ($1/a1$). Because of the high SE and the variability of the SLAR curve one need caution when interpreting the results. In addition, there are also possibly rapid dissociations.

Table 3.1. Models of temporal stability of associations fitted to the dataset ranked by QAIC (Whitehead 1995, 2008a). CC: constant companionships, CA: casual acquaintances.

Models	Components	Parameter estimates and SE	QAIC	ΔQAIC
***a2* + a3*e$^{(-a1*\tau)}$**	**CC + CA**	**a1 = 0.012 ± 0.012** **a2 = 0.007 ± 0.002** **a3 = 0.010 ± 0.003**	**28874**	**0**
*a3*e$^{(-a1*\tau)}$ + a4*e$^{(-a2*\tau)}$	2 levels of CA	a1 = 1.525 ± 3.860 a2 = 0.000 ± 33.660 a3 = 0.059 ± 0.352 a4 = 0.012 ± 2.176	28883	7
*a2*e$^{(-a1*\tau)}$	CA	a1 = 0.001 ± 0.000 a2 = 0.012 ± 0.003	28907	33
a1	CC	a1 = 0.009 ± 0.002	29097	223

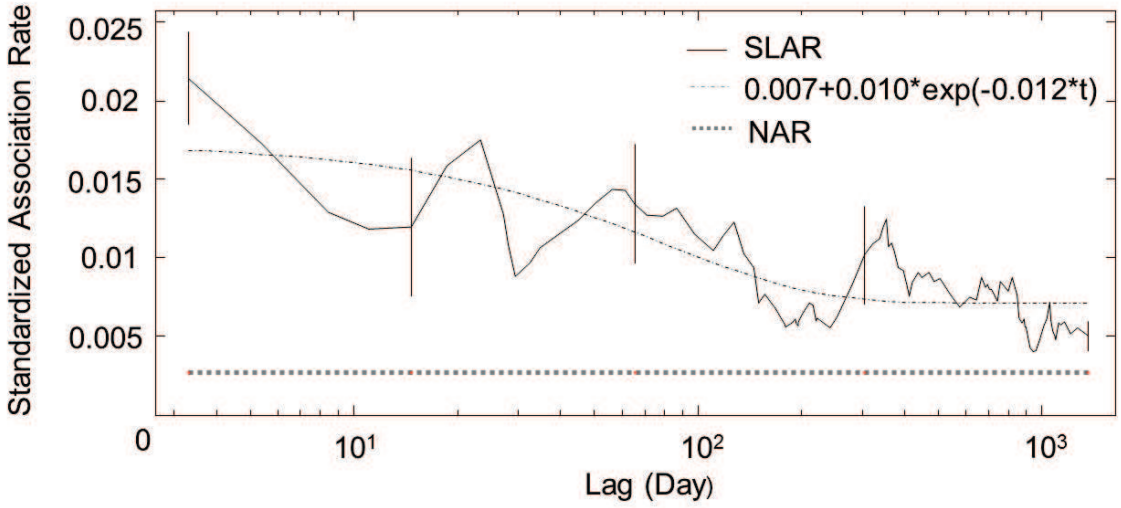


Figure 3.7. Standardized Lag Association Rate (SLAR) for all the individuals is compared to the Null Association Rate (NAR) and the best fitting model (casual acquaintances and constant companions). Error bars were generated by the jackknife technique.

c) Community size

As social clusters were not completely discrete, abundance was estimated for the whole community. The closure assumption was verified according to the Closure Test of Stanley and Burnham (1999) ($P = 0.68$), and the Closure Test of Otis *et al.* (1978) ($P = 0.98$). Model M_t and M_{th} had the smallest AIC_c ($\Delta AIC_c < 2$) and accounted for all the AIC_c weights (Table 3.2). After model averaging, the estimated number of well-marked individuals (M3 and M4) was $N = 124$ (95% confidence interval: 116-141). The mean proportion of well-marked animals on the total number of fins was $\theta = 0.29$ (CV = 0.10) giving an estimated total number of $N' = 420$ dolphins (95% confidence interval: 331-521, SE = 0.11 and CV = 46.92) in 2010.

Table 3.2. Closed population models for abundance estimation ranked by the lowest AIC_c. Model notation: *p*: probability of capture, *c*: probability of recapture, constant parameter: (.), time varying parameter: (*t*), π: mixture parameter, $_A$ and $_B$ refer to the two mixtures, *N*: abundance estimation. SE refers to the Standard Error and *var* to the variance.

Models	**AIC_c**	**ΔAIC_c**	**AICc weigth**	**Model Likelihood**	**Deviance**	***N***	**SE(*N*)**	***var*(*N*)**
$\mathbf{M_t}$ (N, $p(t) = c(t)$)	49.24	0.00	0.67	1.00	118.35	123.10	5.53	30.58
$\mathbf{M_{th}}$ (N, π, $p_A(t) = c_A(t)$, $p_B(t) = c_B(t)$)	50.63	1.38	0.33	0.50	103.18	127.06	6.60	43.56
$\mathbf{M_o}$ (N, p(.) = c(.))	77.45	20.21	0.00	0.00	158.73	124.77	5.87	34.46
$\mathbf{M_h}$ (N, π, $p_A(.) = c_A(.)$, $p_B(.) = c_B(.)$)	81.08	31.84	0.00	0.00	158.33	129.25	11.23	126.11

4) Discussion

a) A fission-fusion social structure

As described in other bottlenose dolphin communities studied so far, the Normano-Breton gulf community lives in a fission-fusion society (Wells *et al.* 1987; Connor *et al.* 2000; Lusseau *et al.* 2006). Associations between individuals were in majority fluid and weak, and in the range of the associations indices observed in other fission-fusion communities (i.e. from 0.06 to 0.2, Wells *et al.* 1987; Smolker *et al.* 1992; Connor *et al.* 2000; Chilvers & Corkeron 2002; Wiszniewski *et al.* 2009). The temporal patterns of associations were also typical of a fission-fusion society where individuals have mainly short-term associates and a smaller proportion of constant companions. Our results also indicated a

gradient in the strength of associations as well as a high variability in relationship durations in the Normano-Breton gulf community. Individuals may therefore adjust grouping patterns according to ecological conditions to maximize fitness gains as observed in other fission-fusion species (e.g. in spotted hyenas Smith *et al.* 2008). Under the general pattern of fission-fusion societies, bottlenose dolphin communities show high variations in relationships among males, females and between males and females at both an inter- and intra-population level (Connor *et al.* 2000; Lusseau *et al.* 2003; Wiszniewski *et al.* 2010b; Connor *et al.* 2011; Wiszniewski *et al.* 2012a). It is likely that differences observed among communities are related to local ecological, breeding, anti-predator constraints and possibly anthropogenic activities which can be highly variable throughout the wide geographical range of the bottlenose dolphin (Lusseau *et al.* 2003; Möller & Harcourt 2008; Augusto *et al.* 2011; Ansmann *et al.* 2012a; Wiszniewski *et al.* 2012b). As found in other communities, stable and high association indices in the Normano-Breton gulf community could indicate male alliances (e.g. Connor *et al.* 1992; Connor *et al.* 1999; Möller *et al.* 2001; Krützen *et al.* 2003; Connor *et al.* 2011). To date, however, alliances have not yet been reported in North-East Atlantic communities (Moray Firth, Scotland and Sado estuary, Portugal, Wilson 1995; Augusto *et al.* 2011). As we excluded individuals with a smooth dorsal fin (M1), which is typical for juveniles, the constant companions likely do not reflect mother and calf bonds in their first years of life. Moderate HWI could indicate female bands (Wells *et al.* 1987; Connor *et al.* 2000; Möller & Harcourt 2008). Associations between males and females are not stable in most communities and tend to be related to reproduction (Connor *et al.* 1992; Smolker *et al.* 1992; Owen *et al.* 2002). In some communities, kin selection (Hamilton 1964) might promote preferential associations with relatives (Krützen *et al.* 2003; Wiszniewski *et al.* 2010b). The influence of the sex and genetic relatedness of the individuals on association patterns in the Normano-Breton gulf will be investigated in the next chapter.

b) Possible ecological drivers of large group sizes

Encountered group sizes (mean = 26) were particularly high and variable (range: 1 to 100) for a resident coastal community. Similar group sizes were observed in highly mobile communities along coastal open habitats (e.g. a mean of 20 individuals along the California coastline, Defran & Weller 1999). However, in contrast to these mobile and wide-ranging

communities, photo-identification work indicated inter-annual site fidelity in the Normano-Breton gulf. Site fidelity is supported by stable isotope data performed on biopsy samples which did not indicate important seasonal trends (see Chapter 4). In most studied resident coastal communities of bottlenose dolphins (*Tursiops* sp.*),* group sizes ranged from 5 to 8 when groups were defined similarly as in our work (Wells *et al.* 1987; Wiszniewski *et al.* 2009; Bouveroux & Mallefet 2010; Ansmann *et al.* 2012a; Fury *et al.* 2013). Caution should however be taken when comparing group sizes as group definition can differ among studies (Connor *et al.* 2000).

Predation risk could not explain these large group sizes, as killer whales or possibly "dolphin-attacking" shark species are not observed in this area and no bite marks were ever recorded in contrast with communities exposed to shark attacks (Heithaus 2001). Delphinids' grouping patterns have also been related to prey availability and/or resource predictability (Lusseau *et al.* 2003; Lusseau *et al.* 2004). In Doubtful Sound (New Zealand), a large mean group size (mean = 17) together with a high proportion of stable associations might allow a high level of cooperation and efficient information transfer in a low productive habitat with scarce resources (Lusseau *et al.* 2003). However, larger group sizes can also be the results of predictable resources. In Moreton Bay (Australia), dolphin groups composed by individuals feeding on trawler discards, a predictable food source, were larger than dolphin groups composed by individuals that did not interact with fisheries (Chilvers & Corkeron 2001; Ansmann *et al.* 2012a). In addition, killer whales (British Columbia) and bottlenose dolphins (Moray Firth, Scotland) groups were smaller in years where less salmon was available (Lusseau *et al.* 2004). We could therefore state two hypotheses for the grouping patterns in the Normano-Breton gulf. First, resources could be scarce and patchy, requiring a high level of cooperation between individuals. However, we would predict more stable and stronger relationships than the ones recorded. The alternative hypothesis is that benefits of grouping patterns (share of knowledge, information exchange, hunting cooperation) could outweigh the costs (feeding competition) as a result of resource availability and/or predictability. Individuals may also adjust grouping patterns according to ecological conditions and behavioral activities as in other fission-fusion species (Wittemyer *et al.* 2005; Smith *et al.* 2008). This flexibility could explain the important variation of group sizes. Additional data, on habitat productivity, ecology and behavior of the dolphins are needed to investigate these hypotheses.

c) Division in three social clusters

We showed that the Normano-Breton gulf bottlenose dolphins were divided in three social clusters. It is important to evaluate whether non-social or indirect social factors could not bias the results when conducting clustering analyses (Cantor *et al.* 2012). Uneven effort could have affected the sighting histories of individuals and separated individuals sighted in different years. However, as the whole area has been surveyed since 2008, we assumed that the partial coverage of the studied area of the first year, and to a lesser extent of the second year of survey, did not greatly affect the clustering results. Moreover, individuals showed inter-annual site fidelity. Therefore, turn-over population factors, as observed for Guiana dolphins, could not account for the division in social clusters (Cantor *et al.* 2012). We investigated whether the clusters were spatially segregated to test if the observed social division could mainly be driven by shared use of space (Lusseau *et al.* 2006). Social clusters showed a degree of spatial segregation since individuals of each cluster were mainly observed in a specific area of the Normano-Breton gulf (i.e. either the northern, the central or the southern part of the gulf depending on the cluster). Mean association indices between individuals of the southern cluster and the northern cluster were particularly low, which indicated a degree of separation (but not isolation) between these clusters. However, ranges of individuals from different clusters largely overlapped, which was expected given the high mobility of dolphins. The observed division in clusters could therefore be linked to a combination of different habitat use and social preferences. These spatial results should be interpreted with great caution as a minimum of five identifications is low to draw conclusions on ranging patterns. Indeed, a minimum of ten to thirty identifications was used in other studies (e.g. Frère *et al.* 2010b; Wiszniewski *et al.* 2012b). The low number of identifications also prevented from using more appropriate methods to estimate the home ranges of highly mobile individuals and core areas, in particular the fixed-kernel density method (Worton 1989), which has been used in social structure studies on delphinids or other mobile species (e.g. Wiszniewski *et al.* 2012b; Best *et al.* 2013; Carter *et al.* 2013). The approach used here is exploratory. Spatial segregation should be further investigated once enough data per individual are gathered, which will allow to use fixed kernel home range analyses. Social division in different clusters is a common feature in bottlenose dolphin societies (Chilvers & Corkeron 2001; Lusseau *et al.* 2006; Wiszniewski *et al.* 2009).

Division into social clusters was linked to ranging patterns in several communities (Lusseau *et al.* 2006; Wiszniewski *et al.* 2009). In the Moray Firth community (Scotland), division is maintained in areas where dolphins of both clusters are observed, indicating that social affiliations are not merely an artefact of habitat use (Lusseau *et al.* 2006). Moreover, even if individuals have distinct ranging patterns, we could not rule out social preferences within clusters. Fine-scale site fidelity can create the opportunity for social preferences to develop, for example, as a result of shared behavioral strategies (Ramos-Fernandez *et al.* 2006; Wiszniewski *et al.* 2009). In other areas, division in social structure may have arisen and may have been maintained in sympatry by different foraging strategies such as interaction or not with fisheries (Chilvers & Corkeron 2001; Ansmann *et al.* 2012a; Daura-Jorge *et al.* 2012) or hunting techniques (e.g. the use of sponges, Mann *et al.* 2012). Multiple others factors such as age, sex and relatedness are also likely to contribute to bottlenose dolphin social affiliations (Möller & Harcourt 2008; Wiszniewski *et al.* 2010b; Mann *et al.* 2012; Fury *et al.* 2013). Here, no interaction with fisheries has yet been reported (GECC, personal communication). Contrary to other communities (Ansmann *et al.* 2012a; Daura-Jorge *et al.* 2012), it is therefore unlikely that variable interactions between bottlenose dolphin groups and fisheries could explain the clustering observed here. However, bottlenose dolphins are known to have various foraging strategies linked to both habitat type and learning during juvenile life (Sargeant & Mann 2009; Torres & Read 2009). Thus, individuals of different clusters may target different prey or feeding habitats. Ecological differences among social groups in the Normano-Breton will be investigated using stable isotope analyses in the next chapter.

d) Abundance

In summer 2010, the estimated abundance over the whole area was 420 (95% CI: 331-521) individuals, making this community one of the largest observed along European coastal waters. In Europe, the size of most coastal communities of bottlenose dolphins ranges from around tens of individuals [Iroise Sea, Brittany, France (Liret 2001); Sound of Barra, Outer Hebrides, Scotland (Grellier & Wilson 2003); Sado estuary, Portugal (Augusto *et al.* 2011)], 100-250 individuals [Moray Firth, Scotland (Wilson *et al.* 1999; Cheney *et al.* 2012); Shannon estuary, Ireland (Berrow *et al.* 2012); Cardigan Bay, England (Pesante *et al.* 2008)] to up to 300-350 individuals [Gibraltar, Spain (Chico Portillo *et al.* 2011)]. Because of uneven

effort, abundance was not estimated for other years (i.e. 2006 – 2009). From now on, the sampling protocol of 2010 should thus be conducted in order to set a long-term demographic monitoring of these dolphins. Once sufficient years of photo-identification surveys in the whole gulf are conducted, the Pollocks' Robust Design (Pollock 1982; Kendall *et al.* 1997) could be an effective method to estimate both abundance, survival and temporary emigration (e.g. Verborgh *et al.* 2009; Daura-Jorge *et al.* 2013).

e) Monitoring and conservation

This study is the first step of a long-term monitoring. It provides important baseline knowledge about the social dynamics and abundance of bottlenose dolphins within the Normano-Breton gulf prior to important anthropogenic activities such as the building of several large-scale marine renewable energy projects. Studies conducted before, during and after the implantation of wind and tide generator farms should enable to assess the long-term consequences of these constructions on this community both in terms of social structure and demography. While the building phase can produce large acoustic disturbances, the sound produced by operating wind turbines is not expected to heavily impact toothed whales (Madsen *et al.* 2006), although studies are lacking on cetaceans other than harbour porpoises (Carstensen *et al.* 2006; Tougaard *et al.* 2009). However, long-term disturbance and slow recovery has been reported in harbour porpoises (Teilmann & Carstensen 2012). Rigorous long-term monitoring of the temporal variations of abundance and distribution, along with demographic parameters such as survival and calving rate, will be invaluable in detecting the effects of future human activities on this community. Moreover, the persistence of the social clusters and their ranging patterns should also carefully be monitored. As suggested by Lusseau *et al.* (2006), if social clusters show clear spatial or ecological segregation, models of population dynamics could take the social division into account as co-variates. In addition, as detailed in the introduction, social structure is likely driven by environmental factors. Thus, changes in the environment, for instance on the distribution and abundance of resources, could impact social structure (Blumstein 2012). Monitoring long-term social dynamics in the future will therefore help to understand eventual population responses to changes in ecological conditions (Parsons *et al.* 2009; Blumstein 2012; Foster *et al.* 2012).

Given the high abundance, and inter-annual site fidelity of bottlenose dolphins in the gulf, we suggest that a Special Area of Conservation should be designated for these dolphins. Habitat use analyses would be needed to spatially delineate the conservation area. Moreover, bottlenose dolphin is one of the main year-round top-predator in the gulf along with harbour seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*) and seabirds (GECC, personal communication). The monitoring of bottlenose dolphins could therefore be used as a bio-indicator of the Normano-Breton gulf ecosystem health (Hooker & Gerber 2004). Finally, the factors shaping the social structure of this community will be investigated in the next chapter using genetic and stable isotope analyses.

CHAPTER

4

EVALUATING THE INFLUENCE OF ECOLOGY, KINSHIP AND PHYLOGEOGRAPHY ON THE SOCIAL STRUCTURE OF RESIDENT COASTAL BOTTLENOSE DOLPHINS

1) Introduction

Animal social structures are shaped by the trade-off between the benefits and costs of group living (see review in Krause & Ruxton 2002). While sociality can provide benefits such as increased foraging efficiency (e.g. Packer & Ruttan 1988), knowledge sharing (e.g. McComb *et al.* 2001; Safi & Kerth 2007) and reduced predation risk (e.g. Kelley *et al.* 2011), it can also incur costs such as competition for food resources or mating, and disease transmission (Wrangham *et al.* 1993; Clutton-Brock *et al.* 1998; Altizer *et al.* 2003; Clutton-Brock 2007). Environmental variability can modify the costs and benefits of living in groups, leading to intraspecific or intra-population variations in social organization. For instance, resource availability or foraging techniques can lead to intraspecific social behavior variations (e.g. Karczmarski *et al.* 2005; Chaverri 2010; Beck *et al.* 2012). The same factors, in addition to seasonal changes, can modify a given population' social structure (e.g. seasonal changes in food resources for elephants, Wittemyer *et al.* 2005; prey availability for spotted hyenas, Smith *et al.* 2008; the loss of an anthropogenic food resource for bottlenose dolphins, Ansmann *et al.* 2012a; or salmon abundance for killer whales, Foster *et al.* 2012). These extrinsic factors act in interaction with intrinsic behavioral factors. Individuals may prefer to associate with conspecifics with whom they share similar characters. Homophily can be based on age (e.g. Wey & Blumstein 2010), sex (see review in Ruckstuhl 2007), kinship (Hamilton 1964; Holekamp *et al.* 1997; Wiszniewski *et al.* 2010b), reproductive condition (e.g. Möller & Harcourt 2008) or behavioral phenotypes (e.g. Croft *et al.* 2009; Mann *et al.* 2012). Social structure may have evolutionary impacts by influencing patterns of gene flow (Piertney *et al.* 1999; Storz 1999; Pilot *et al.* 2010).

In fission-fusion societies, although individuals may maintain long-term bonds with specific companions, associations are mainly temporary and show hourly or daily turn-overs. This highly flexible social organization can be strongly influenced by ecological factors (Couzin 2006; Lehmann *et al.* 2007; Smith *et al.* 2008). Bottlenose dolphin (*Tursiops* sp.) societies are fission-fusion (Connor *et al.* 2000). They are found in a wide range of environments from tropical to temperate areas, and shallow inshore enclosed estuaries to deep pelagic waters. Hence, large behavioral variations conditioned by ecological selection can be expected. Male mating strategies and social behavior vary both between and within

populations. In inshore *Tursiops* sp. and *T. aduncus* populations of Australia and inshor*e T. truncatus* populations of the North-West Atlantic, males form alliances of varying degree of complexity, both between related and unrelated individuals, to compete for females (Krützen *et al.* 2003; Parsons *et al.* 2003; Connor *et al.* 2011; Wiszniewski *et al.* 2012a). In contrast, they do not seem to form alliances in the North-East Atlantic coastal population of Moray Firth (Wilson 1995). Females tend to form "bands" and share moderate bonds with related and unrelated females (Wells 1991; Frère *et al.* 2010b; Wiszniewski *et al.* 2010b) or with females in the same reproductive state (Möller & Harcourt 2008). Male and female relationships seem to be linked to reproduction (Smolker *et al.* 1992; Owen 2003). However, in a New Zealand fjord, the scarcity of resources, probably requiring a higher level of cooperation, is thought to have shaped stable mixed-sex groups (Lusseau *et al.* 2003). In addition, bottlenose dolphin feeding behavior, which is shaped by environmental characteristics (Torres & Read 2009) and learning during juvenile life (Sargeant & Mann 2009), is also highly plastic. Shared behavioral strategies such as foraging techniques can also influence social organization (Ansmann *et al.* 2012a; Mann *et al.* 2012). Their complex social structure, together with ecological specializations, may have implications on gene flow and could lead to fine-scale population structure (Sellas *et al.* 2005; Ansmann *et al.* 2012b).

The influence of ecology on social structure has been studied through direct observations of feeding behavior (Ansmann *et al.* 2012a; Mann *et al.* 2012). However, feeding specializations cannot always be observed visually, especially in temperate seas where water is generally not clear enough to monitor underwater behavior from the boat. In that context, stable isotopes such as sulfur ($\delta^{34}S$), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can provide indirect information of a consumer forage resources (see Chapter 2.1.b for more details on stable isotope analyse principles). Sulfur and carbon stable isotopes are indicators of feeding habitats and can separate inshore *vs.* offshore and pelagic *vs.* benthic food resources (Peterson & Fry 1987; Kelly 2000; Connolly *et al.* 2004). $\delta^{34}S$ values varies from 2 to 6‰ in terrestrial habitats to 21‰ in marine habitats (Peterson & Fry 1987). Stable isotopes of nitrogen are enriched in tissues of consumers relative to their food resources, and they can therefore provide information on consumer trophic position (Kelly 2000). They can also be used to discriminate between different habitats (e.g. pelagic *vs.* coastal, Chouvelon *et al.* 2012).

Here, we focus on bottlenose dolphins in coastal waters of the English Channel which are part of the North-East Atlantic coastal genetic ecotype (Chapter 5). In the Normano-Breton gulf, three social clusters have been identified in the previous chapter through social structure analyses based on photo-identification data. The animals formed large groups in comparison with other resident coastal bottlenose dolphins. In this chapter, we tested whether social behavior influenced gene flow using Bayesian clustering analyses on microsatellite markers and by examining mitochondrial haplotype frequencies. We then assessed if the three social clusters were ecologically distinct using stable isotope signatures of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$. Finally, the relative influence of sex, genetic relatedness and ecological similarity on association patterns was investigated. Stable isotope signatures were used as a proxy for foraging ecology and hence ecological homophily. We compared the social drivers of this coastal open-water population with other populations inhabiting various habitats and discussed the ecology and evolutionary processes that are likely to drive sociality.

2) Material and methods

a) Boat surveys, biopsy sampling and photo-identification

98 biopsy samples were collected during boat surveys from September 2010 to August 2012 using a crossbow (Panzer Barnett 5) and tips and arrows made by Finn Larsen (Danish Institute for Fisheries Research, see Figure 4.1a in the result section for sampling locations). Individuals were photo-identified (i.e. using the natural marks on their dorsal fins) at the time of sampling. Prior to sampling, they were also identified visually to avoid double sampling as much as possible. We only sampled adults, excluding mothers that had a calf of less than two years. Samples contained both skin and blubber tissues and were generally 6 mm in diameter and 1.5 cm long. Biopsy samples were collected under the permit 09/115/DEROG from the French ministry. Skin samples were frozen at -20°C.

b) Social structure

The social structure of this population was analyzed in the previous chapter using photo-identification data collected between 2006 and 2010. In short, pairwise association coefficients (HWI: Half-Weight Index) were calculated between pairs of individuals sighted in at least five different days using SOCPROG (N = 213) (Whitehead 2009b). Using the dendrogram-based modularity method, three social clusters ("South", "Minquiers" and "North" named according to the areas where the individuals were in majority observed, see Figure 4.1a for their locations) have been identified and each individual was assigned to one of the social clusters (see Chapter 3 for details on social structure analyses). Fifty-four biopsy-sampled individuals were included in social structure analyses. The remaining sampled individuals were either sighted in less than five different days (N = 24) or were not identified either because they were unmarked or because the quality of the photo taken was not good enough to recognize the individuals (N = 12). Eight individuals were sampled twice.

c) Genetic analyses

DNA was extracted using NucleoSpin Tissue kits (Macherey-Nagel) following the manufacturer's protocol. 92 samples were genotyped at 25 microsatellite loci including 20 published markers and 5 markers newly-developed during this study (see Chapter 2.1.d for general information on microsatellite markers; Appendix A4.1 for PCR, genotyping conditions and the characteristics of the microsatellite loci; Appendix A4.2 for the method of discovery of new microsatellites and Appendix A4.3 for microsatellite marker characteristics for the studied individuals). Six samples out of the 98 biopsies were excluded from the analyses as they were duplicates according to photo-identification at the time of sampling. Two possible duplicates were included in the analyses to genetically confirm their identity. A 682 base-pair (bp) portion of the mitochondrial control region was amplified and sequenced for all samples using primers Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') (Baker *et al.* 1998) and Dlp8G (5'-GGAGTACTATGTCCTGTAACCA-3') (as reported in Dalebout *et al.* 2005). Amplification and sequencing conditions are available in Appendix A4.4 and general characteristics of mitochondrial markers are given in Chapter 2.1.b. Individuals were sexed using the SRY plus ZFX/ZFY fragments amplification method described in Rosel (2003).

Microsatellite marker quality

To evaluate genotyping error rate, nine individuals were randomly selected for re-amplification and scoring at all loci. The two duplicates, which were confirmed using the program Excel MicrosatelliteToolkit (Park 2001), were also included in error rate calculation. Ten percent of the dataset was thus re-analyzed. All individuals were successfully amplified for at least 23 loci and there was 0.56% of missing values in the dataset. Microchecker 2.2.3 was used to check for null alleles* and scoring errors (Van Oosterhout *et al.* 2004). Departures from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium were tested using 10 000 dememorizations, 1 000 batches and 10 000 iterations per batch in GENEPOP on the web version 4.2 (Raymond & Rousset 1995; Rousset 2008). Significance levels were corrected for multiple comparisons using the sequential Bonferroni technique (Holm 1979).

Mitochondrial DNA sequences

We generated consensus sequences for the 682-bp portion of the mitochondrial control region and looked for ambiguities with Sequencher 5.0 Demo (Gene Codes Corporation). Sequences were then manually edited with BioEdit 7.1.3.0 (Hall 1999). Unique haplotypes were identified using DNAsp 5 (Rozas & Rozas 1999).

d) Genetic population structure

Microsatellites

Three clustering methods were applied to microsatellite data of all individuals (N=90) to determine the most likely number of populations and assign individuals to these: two Bayesian methods implemented in STRUCTURE (Pritchard *et al.* 2000) and TESS (Durand *et al.* 2009b) and a multivariate method, the Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.* 2010). These three different approaches were used to ensure the robustness of the inferred results as determining the most likely number of clusters can be challenging (Guillot *et al.* 2009). STRUCTURE assigns individuals to clusters by minimizing Hardy-Weinberg and linkage disequilibria (Pritchard *et al.* 2000). TESS implements a

spatially explicit Bayesian model, which incorporates the geographic coordinates of the sampled individuals as *a priori* information (Durand *et al.* 2009b). The DAPC uses genetic similarity to cluster individuals and does not make any population genetic model assumptions (Jombart *et al.* 2010, see Chapter 2.2.b for more details on these three methods).

In STRUCTURE, the admixture models with correlated and uncorrelated allele frequencies were run, without indicating any *a priori* information. Ten independent runs for each *K* value from 1 to 10 were carried out with a burnin-period of 50 000 iterations followed by 300 000 Markov Chain Monte Carlo (MCMC) steps. To determine the most likely number of clusters, we plotted Ln*P*(*D*) (Pritchard *et al.* 2000), calculated ΔK (Evanno *et al.* 2005) in STRUCTURE Harvester v.0.5 (Earl & Vonholdt 2012), and examined individual membership proportion[2] plots as well as the consistency across runs.

The conditional auto-regressive (CAR) admixture model was run in TESS using a burnin of 20 000 steps followed by 120 000 MCMC steps. The number of clusters (*K*) to test was set from 2 to 10 and 10 replicate runs for each *K* were performed. Default parameters of the model were used: a spatial interaction parameter of 0.6 and a linear degree of trend. To select the most likely number of clusters, we plotted Deviance Information Criterion (DIC) values against *K* and examined plots of individual membership proportions. We also checked the consistency across runs. TESS does not test for $K = 1$, although it could be examined using the plots of individual membership proportions (i.e. if for $K = 2$ all individuals show membership proportions superior to 0.8 for the same cluster, it can be considered that the most likely number of populations is 1).

DAPC was performed using the package adegenet 1.3.6 (Jombart 2008) in R 3.0.0 following the recommendations of Jombart (2012). The most likely number of clusters was determined with a *K*-means method using the lowest BIC (Bayesian Information Criterion) value and the elbow in the BIC curve. Maximum number of clusters was set to 40 and all the principal components (PCs) were retained. In the DAPC, the genetic data were first transformed using Principal Component Analysis. Then, a linear discriminant analysis was performed on the retained PCs (in order to maximize genetic variation between clusters and

[2] For vocabulary simplification we use "individual membership proportions" to refer to the percentages of the genome of an individual that came from each population (i.e. admixture proportions, see Chapter 2.2.b for details).

minimize it within clusters). We retained 80% PCs to avoid over-fitting as well as all eigenvalues.

The inclusion of closely related individuals can affect population structure analyses (Anderson & Dunham 2008). Therefore the Queller and Goodnight (Queller & Goodnight 1989) relatedness coefficient (*R*) was estimated among individuals using KINGROUP v.2 (Konovalov *et al.* 2004). Finally, STRUCTURE, TESS and DAPC were re-run after removing one individual from each pair of individuals showing a relatedness coefficient superior or equal to 0.45 as in Rosel *et al.* (2009).

Mitochondrial DNA

A haplotype network was constructed with the median-joining and maximum-parsimony algorithms implemented in Network 4.6.0.0 (Bandelt *et al.* 1999). Haplotypes for each sample (N = 90) were displayed on a map created using the marmap package version 0.7 (Pante & Simon-Bouhet 2013) in R 3.0.0 (R Core Team 2013) and haplotype frequencies per social cluster were also represented (N = 54).

e) Ecological population structure

Stable isotopes of sulfur ($\delta^{34}S$), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were analyzed in skin samples (N = 88, as for two samples we did not have enough skin for both genetic and stable isotope analyses). Prior to isotopic analyses, skin samples were cut in microscopic pieces and dried at 45°C in an incubator for 48 h. As lipids are depleted in ^{13}C relative to other tissue components (De Niro & Epstein 1977), they were extracted from skin samples prior to stable isotopes analyses (SIA) of carbon and nitrogen but not of sulfur. Lipid extraction had no effect on stable isotope values of sulfur: differences between measurements with lipid extraction and without lipid extraction were less than 0.2‰, which is in the precision range of the measurements. Sampled powders were agitated with 2 ml of cyclohexane for 1 h and centrifuged for 10 min at 3500 tours/min. Supernatants containing lipids were discarded. This protocol was repeated until the supernatant were transparents. Samples were dried in an incubator for 48 h. Subsamples were weighted (0.3 – 0.4 mg for

carbon and nitrogen SIA and 1.0 - 1.3 mg for sulfur SIA) with a microbalance and packed in tin cups. Sulfur, carbon and nitrogen isotope ratios were determined by a continuous flow mass spectrometer (Thermo Scientific, Delta V Advantage) coupled to an elemental analyzer (Thermo Scientific, Flash EA 1112). Stable isotope values are presented in the conventional δ notation (in ‰) relative to IAEA-1 and IAEA-2 for $\delta^{34}S$ values, Vienna Pee Dee Belemnite for $\delta^{13}C$ values and atmospheric N2 for $\delta^{15}N$ values. Isotopic measurement errors were less than 0.20‰ for $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$. To ensure that the lipid extraction was effective, we verified that the C/N mass ratios of all the samples were below 4.

All stable isotope statistical analyses were carried out in R 3.0.0. Mean differences between the three dolphin social groups and between males and females' $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$ values were compared using Student *t*-tests or Mann–Whitney-Wilcoxon tests (depending on whether the data satisfied the required conditions: normality and homogeneity of variances). Significance levels were adjusted for multiple comparisons using the sequential Bonferonni method. Seasonal variations in stable isotope values were also evaluated.

Stable isotope niches of the three social groups were estimated using multivariate, ellipse-based metrics: SIBER (Stable Isotope Bayesian Ellipses in R, Jackson *et al.* 2011) implemented in the SIAR package version 4.2 (Parnell & Jackson 2011). Standard ellipse is to bivariate data what standard deviation is to univariate data. The standard ellipse area (SEA) is defined by a subsample (40%) of the bivariate data (in our case, the ratios of $\delta^{34}S$ and $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ and $\delta^{13}C$ and $\delta^{15}N$) and was calculated from the variance and covariance of the data. We corrected SEA for sample size (SEA_c). This approach is robust when comparing small and unbalanced sample sizes and is not biased by outliers (Jackson *et al.* 2011). The degree of SEA_c overlap between each social cluster was also estimated.

To test for subdivision in the dataset, a clustering analysis was performed based on probabilistic models with no *a priori* using mclust package version 4.2 (Fraley & Raftery 2002; Fraley *et al.* 2012). It implements a maximum-likelihood clustering approach based on Gaussian mixture models. Model parameters are estimated using the Expectation Maximization (EM) algorithm initialized by hierarchical model-based clustering. The default settings were used where the optimal model (out of 10 models with different covariance structure) and number of clusters (set from 1 to 9) were selected by BIC (Bayesian Information Criterion). The analysis was performed both for the whole dataset and only for

individuals whose social group is identified. For the latter, assignments obtained were compared to the social cluster assignments.

f) Influence of relatedness, sex and ecology on association patterns

To test whether genetic relatedness, maternal kinship, similar ecology, and sex were significant predictors of the strength of associations, a Double Dekker Semi-Partialing Multiple Regression Quadratic Assignment Procedure (MRQAP) was carried out using the sna package 2.3.1 in R 3.0.0. (Dekker *et al.* 2007; Butts 2013). The MRQAP is an extension of the Mantel test that allows a dependent matrix to be regressed simultaneously against multiple explanatory matrices that represent dyadic attribute relationships. Its interpretation is similar to multiple regression, but it takes non-independence of the pairwise data into account by randomly permuting the dependent matrix (see Mann *et al.* 2012 and Wey & Blumstein 2010 for further details). Association indices (Half-Weight Index) were the response matrix. Bi-parental relatedness, maternal kinship, sex homophily and ecological similarity were the explanatory matrices.

Pairwise relatedness values were estimated as described earlier using the Queller and Goodnight (1989) relatedness coefficient (*R*). All individuals were used to calculate allele frequencies (N = 90), and then *R* was calculated between individuals used in social structure analyses (N = 54). For the maternal kinship matrix, dyads having the same haplotypes received a 1 and dyads having different haplotypes a 0. Male and female homophily matrices were created by assigning a value of 1 if dolphins were of the same sex and 0 otherwise. For the ecological similarity matrix, Euclidean distances of the values of δ^{34}S, δ^{13}C and δ^{15}N (*distISO*) between individuals (*i* and *j*) were first calculated as follows:

$$distISO = \sqrt{(\delta^{34}Si - \delta^{34}Sj)^2 + (\delta^{13}Ci - \delta^{13}Cj)^2 + (\delta^{15}Ni - \delta^{15}Nj)^2}.$$

Then, the similarity matrix was calculated by subtracting *distISO* from the maximum of *distISO*.

Mantel tests were conducted to evaluate the influence of each matrix on the association matrix using 10 000 permutations and the ade4 package 1.3.6. (Chessel *et al.* 2004; Dray & Dufour 2007; Dray *et al.* 2007).

All analyses were carried out for the whole dataset and for males and females separately. Unless otherwise notified, results were similar to the ones obtained with both sexes.

We tested whether relatedness was higher within social clusters than expected at random using a randomization test in R.3.0.0. Mean relatedness was calculated for each social cluster. Individuals were randomly permuted 10 000 times between groups. The number of individuals was kept identical as in the observed dataset. Significance was assessed by comparing the distribution of permuted mean relatedness for each cluster to the observed mean relatedness. For mtDNA data, we tested whether individuals were more likely to share mitochondrial DNA haplotypes within social clusters than expected at random using a similar randomization test. The sum of dyads sharing haplotypes was calculated for each social cluster. Individuals were randomly permuted 10 000 times between groups. The number of individuals was kept identical as in the observed dataset. Significance was assessed by comparing the distribution of the permuted sums of dyads matching haplotypes for each cluster to the observed sum.

3) Results

a) Biopsy sampling

Biopsy samples were obtained from 90 different individuals including 28 females and 62 males. 54 individuals were included in social structure analyses and were composed of 39 males and 15 females. The dataset was therefore clearly male biased. We avoided sampling mothers with calves and were thus less likely to sample females than males. In addition, this bias could also be linked to possible differential behavior reactions towards the boat between males and females.

b) Genetic population structure

No significant departure from HWE and no null alleles were detected. Linkage disequilibrium was significant for 0.7% of the pairwise comparisons and was therefore considered negligible. The genotyping error rate was 0.0036 (i.e. 1 incorrect genotype / 275 genotypes reprocessed).

The most likely number of populations was one using DAPC and TESS (see DAPC BIC plot and TESS barplot for K=2 in respectively Appendix A4.5 and A4.6a). Identical results were obtained when one individual per pair of closely related individual was removed (i.e. 18 individuals). Although the most likely number of clusters was not straightforward when examining the STRUCTURE Ln$P(D)$ plot (Appendix 4.7a) for the whole dataset, the examination of the membership proportion plots indicated that there was only one population (Appendix A4.6b). Moreover, when removing one individual per pair of close relatives, both membership proportion and Ln$P(D)$ plots indicated that the most likely number of population was one (Appendix A4.6c and A4.7b). Models with uncorrelated and correlated allele frequencies produced similar results.

Five haplotypes were identified in the dataset (GENBANK accession numbers from KF650783 to KF650787 for haplotypes 1 to 5, respectively). The median-joining network indicated that the majority of individuals shared three haplotypes that are separated by 1 bp (Figure 4.1b). However, two males had more distant haplotypes (separated by 11 pb from the main lineage). The three main haplotypes were shared by both males and females (Figure 4.1c). We did not detect any spatial organization of the haplotypes nor major differences in haplotype frequencies between social clusters (Figure 4.1a and 4.1d).

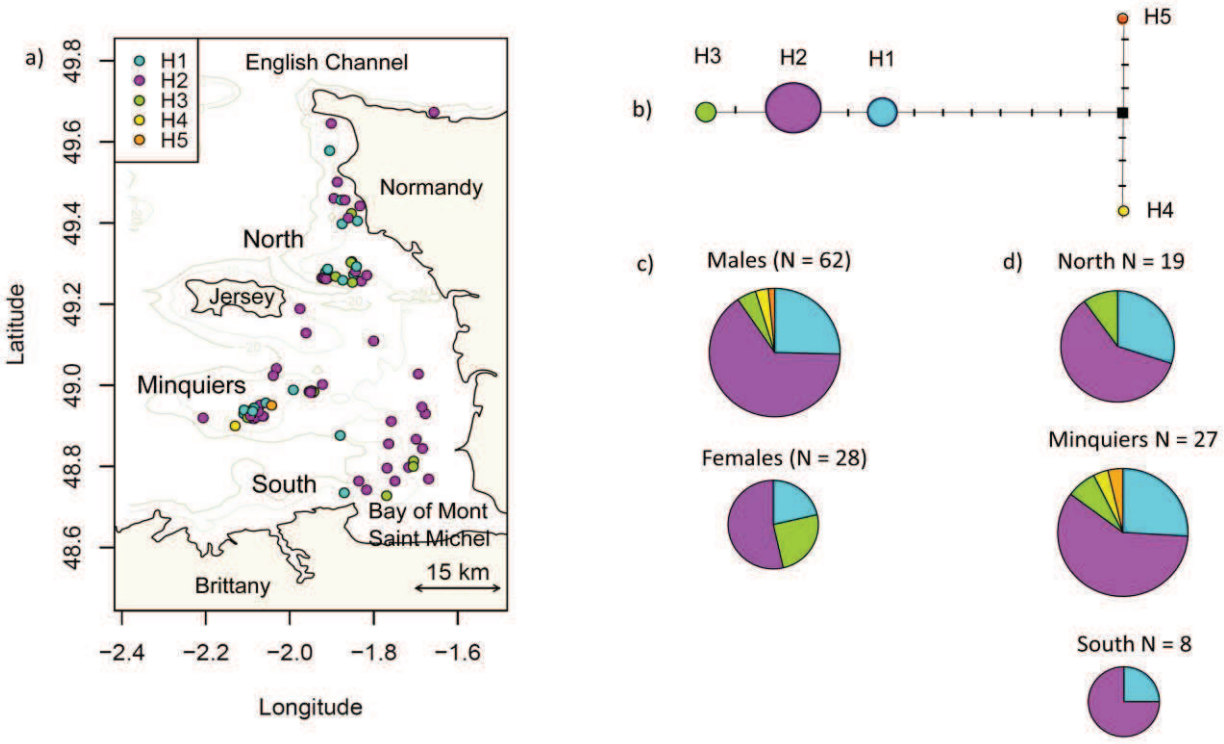


Figure 4.1. Mitochondrial DNA results for the Normano-Breton gulf bottlenose dolphins. a) Map showing the haplotype of each biopsied bottlenose dolphin whether they were included in the social structure analyses or not (N = 90). b) Median-joining network of mitochondrial DNA control region haplotypes found in bottlenose dolphins from the Normano-breton gulf. The size of the circles is proportional to haplotype frequencies. Black squares indicate either extinct or unsampled intermediate haplotypes. Black dashes indicate mutation steps between haplotypes. c) Piecharts of haplotype frequencies for each sex. b) Piecharts of haplotype frequencies for each social cluster (N = 54).

c) Ecological population structure

$\delta^{34}S$ values were significantly different between Minquiers and South, and between Minquiers and North social clusters ($P < 0.01$, Table 4.1, see Figure 4.1 for area locations and Chapter 3 for social cluster details). For $\delta^{13}C$ and $\delta^{15}N$, differences were only significant between Minquiers and North clusters ($P = 0.01$ and $P < 0.01$ respectively).

Table 4.1. Stable isotope values (mean +/- SD) for each social cluster (‰).

Social cluster	N	$\delta^{13}C$	$\delta^{34}S$	$\delta^{15}N$
South	8	-17.2 ± 0.4	14.9 ± 1.0	14.9 ± 0.4
Minquiers	27	-17.3 ± 0.4	16.0 ± 0.4	14.6 ± 0.5
North	19	-16.9 ± 0.4	15.5 ± 0.6	15.2 ± 0.3

There were no significant differences between males or females in stable isotope values or major seasonal trends (see Appendix A4.8a to A4.8c for variations in stable isotope values according to season).

SEA_c for $\delta^{13}C$ and $\delta^{15}N$, and $\delta^{34}S$ and $\delta^{13}C$ overlapped between all social clusters (Table 4.2, Appendix A4.9a and A4.9b). SEA_c for $\delta^{34}S$ and $\delta^{15}N$ showed little spatial overlap (Figure 4.2, Table 4.2). Given the above results, we considered only $\delta^{34}S$ and $\delta^{15}N$ values for the estimation of the most likely number of clusters with no *a priori.* The estimated number of clusters was 3 with 70% of the individuals assigned to the same stable isotope group as their social group. It should be noted that the sample size for the South cluster (N = 8) was relatively limited.

Table 4.2. Areas of overlap between SEA_c of different social cluster pairs (‰²).

Pair of SEA_c	$\delta^{13}C$ and $\delta^{34}S$	$\delta^{34}S$ and $\delta^{15}N$	$\delta^{15}N$ and $\delta^{13}C$
South and Minquiers	0.08	0.04	0.29
South and North	0.40	0.01	0.09
North and Minquiers	0.07	0.09	0.11

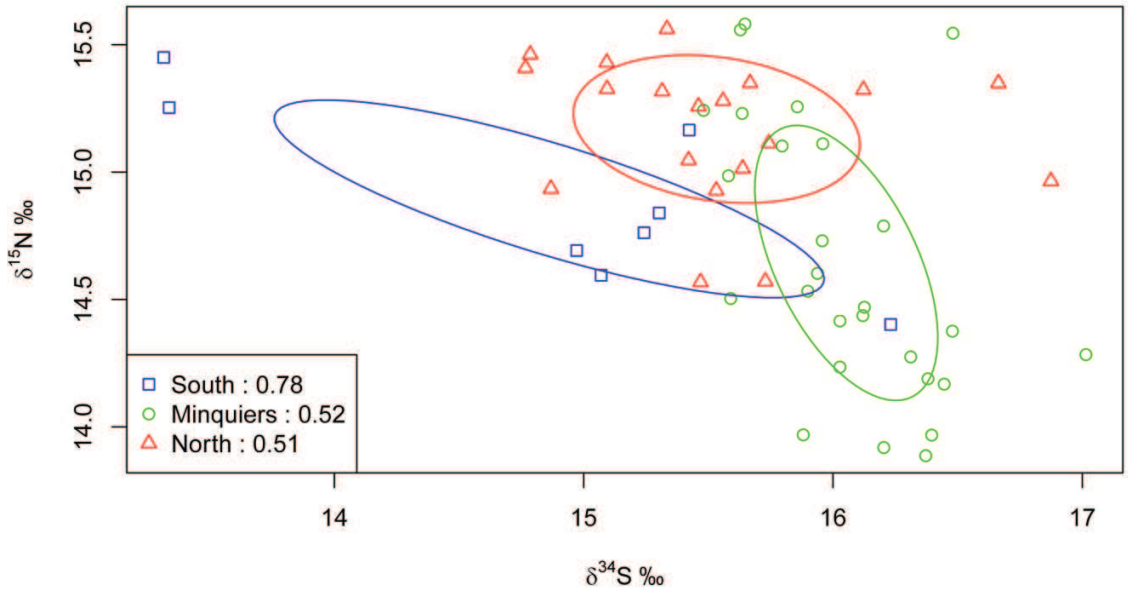


Figure 4.2. $\delta^{34}S$ and $\delta^{15}N$ signatures for each social group of bottlenose dolphins. Solid lines indicate Standard Ellipses Areas corrected for small sample sizes (SEA_c). Area values are given in the legend (‰²).

d) Influence of relatedness, sex and ecology on association patterns

Ecological similarity and maternal kinship were the only significant predictors of association strengths both when conducting MRQAP (Table 4.3) and Mantel tests. Only 5% of the variance in HWI was explained by these two variables in the MRQAP analysis. The effect of ecological similarity was positive while the effect of maternal kinship was negative.

Table 4.3. Results from the MRQAP analysis. Significant *P*-values ($P < 0.05$) are indicated in bold.

Variable	Unstandardized coefficient	*P*-value
Ecological similariy	**0.05**	**0.00**
Biparental relatedness	0.03	0.21
Maternal kinship	**-0.03**	**0.01**
Female homophily	0.03	0.10
Male homophily	-0.00	0.69

When removing maternal kinship, the variance explained did not change substantially (0.7%), indicating that this variable had little influence on HWI. This was confirmed using a Mantel test for which the observed correlation was r = - 0.07 between maternal kinship and HWI matrices (P = 0.01). In contrast, the observed correlation was r = 0.19 between ecological similarity and HWI matrices (P < 0.01, Figure 4.3). Strongly associated individuals had similar ecology while weakly or never associated individuals may have similar or contrasted ecology (Figure 4.3). Sex and bi-parental relatedness had no influence on HWI (Table 4.3, Mantel tests P = 0.45 and P = 0.09 respectively, Figure 4.4). There were strong associations both between males (number N of pairs showing a HWI ≥ 0.5 = 24), females (N = 7) and between males and females (N = 25), although it should be noted that a limited number of females were sampled. In these strong association pairs, we found only one pair of first-order relatives (R > 0.45) between two males.

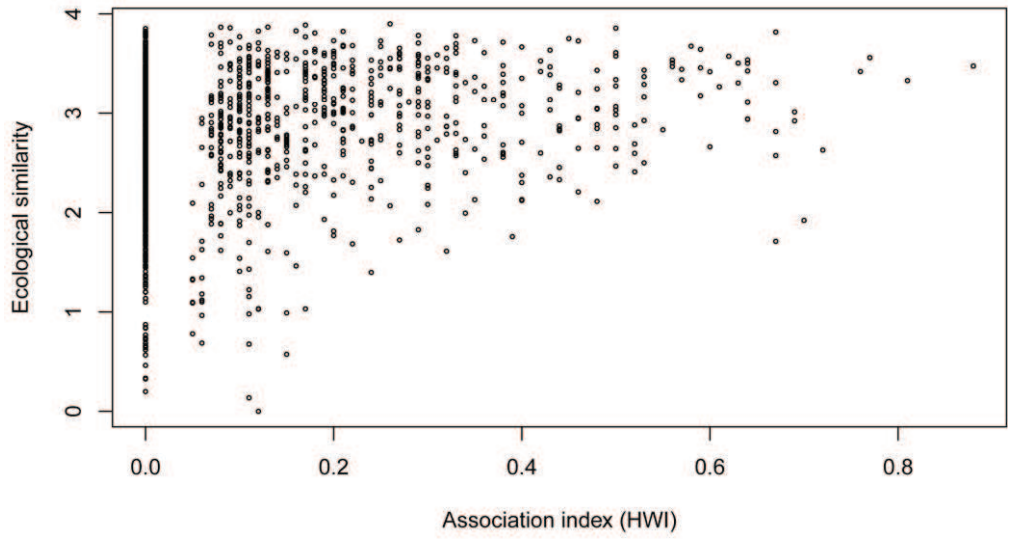


Figure 4.3. Relationship between ecological similarity and association index for each pair of bottlenose dolphins in the Normano-Breton Gulf, English Channel (N = 54 individuals; 1431 pairs).

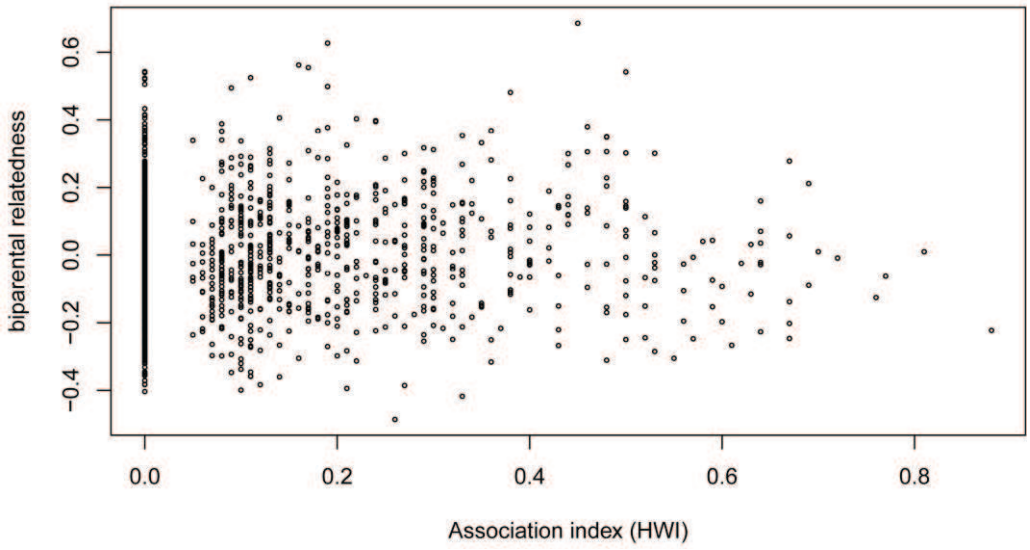


Figure 4.4. Relationship between biparental relatedness and association index for each pair of bottlenose dolphins in the Normano-Breton Gulf, English Channel (N = 54 individuals; 1431 pairs).

Permutation tests indicated that mean relatedness observed within social clusters was not higher than expected at random (Table 4.4). In addition, individuals were not more likely to share haplotypes within each social cluster than expected at random (Table 4.5).

Table 4.4. Results of the permutation test to evaluate whether the mean observed relatedness (*R* observed) within each social cluster is higher than the mean relatedness generated using permutations (*R* random) for each social cluster. There are no significant *P*-values after Bonferroni correction (significant values at the 5% threshold are those for which $P < 0.017$).

Social cluster	*R* observed	*R* random	*P*-value
Minquiers	0.023	-0.001	0.05
South	0.039	-0.000	0.17
North	-0.016	-0.001	0.78

Table 4.5. Results of the permutation test to evaluate whether the observed sum of dyads matching haplotypes within each social cluster (*S* observed) is higher than the sums of dyads matching haplotypes in randomly generated data (*S* random).

Social cluster	*S* observed	*S* random	*P*-value
Minquiers	142	155	0.70
South	16	13	0.10
North	71	77	0.61

4) Discussion

a) Three social and ecological clusters but a single population

We showed in the previous chapter that bottlenose dolphins in the Normano-Breton gulf were divided in three social clusters. Here, we found good consistency between social structure and stable isotope clustering analyses. The three social clusters were ecologically distinct and sulfur stable isotopes were particularly efficient at detecting differences among groups. We previously suggested that the three social clusters were spatially segregated despite some overlap (Chapter 3). Here, $\delta^{34}S$ results were consistent with the main sighting areas of the individuals (see Chapter 3 for details). $\delta^{34}S$ values are increasing from terrestrial habitats (2 to 6‰) to marine habitats (21‰, Peterson & Fry 1987). Individuals of the Minquiers social cluster, which is the farthest area from shore where we observed dolphins, showed higher values of $\delta^{34}S$ than the dolphins from the North and South clusters. In contrast, individuals of the South cluster, mainly sighted in and near the Bay of Mont Saint Michel (an estuary), had the lowest $\delta^{34}S$ values. This study highlighted the power of $\delta^{34}S$, in addition to $\delta^{13}C$ and $\delta^{15}N$, to investigate population structure and ecology of marine top predators as it was shown for yellow-eyed gulls (Moreno *et al.* 2010) and bottlenose dolphins in Florida (Barros *et al.* 2010; Olin *et al.* 2012).

In contrast, there was no genetic structure, which is not surprising given the high mobility of the species and the small size of the surveyed area. However, fine-scale genetic structure has been observed in bottlenose dolphins or other delphinids in areas of similar size to the Normano-Breton Gulf (around or less than 100 km), possibly as a result of social structure and ecology (e.g. Wiszniewski *et al.* 2010a; Hollatz *et al.* 2011; Ansmann *et al.* 2012b). In the Normano-Breton Gulf, social clusters are not discrete, i.e. all individuals are indirectly connected to each other (Chapter 3). This inter-connected social network together with the small size of the area, the continuous environment (in contrast to separate bays) could explain the absence of genetic structure. Another hypothesis could be that if there is any genetic structure, it could be too recent to be detected with the set of markers used in this study. Indeed, the different approaches used provide information about population processes at different time scales. Molecular markers informed us on evolutionary time scales, with microsatellites integrating more recent events (a few generations) than mitochondrial sequences. Social structure analyses resulted from photo-identification data collected between 2006 and 2010. Skin stable isotope values should be representative of the diet of at least two months considering a turn-over of 73 days for bottlenose dolphin skin (Hicks *et al.* 1985). However, a recent study using experiments on captive individuals found a retention time of 20 to 32 days for $\delta^{13}C$ and $\delta^{15}N$ in skin (Browning *et al.* 2014). Biopsy sampling only partially overlapped in time with the photo-identification data. Nonetheless, stable isotope clustering results that are representative of the diet of individuals over the past few weeks were consistent with social structure results collected over several years.

b) Ecology but not kinship influences social structure

Kin selection theory predicts that associating with kin can provide indirect fitness benefits and higher survival, reproductive output and food intake (Hamilton 1964; Alexander 1974; Silk 2007; Frère *et al.* 2010a). Here, we did not find any influence of relatedness on social structure, which was thought to be the norm for at least some female associations in inshore bottlenose dolphin societies (Frère *et al.* 2010b; Wiszniewski *et al.* 2010b) and in most fission-fusion species such as giraffes (Carter *et al.* 2013), spotted hyenas (Holekamp *et al.* 1997) and elephants (Archie *et al.* 2006). However, we had a limited sample size of females. Males did not associate preferentially with kin either. In inshore-water populations of

Australia and the North-West Atlantic (Sarasota Bay, Florida), males formed various types of alliances to compete for females (Owen *et al.* 2002; Connor *et al.* 2011). These alliances can occur between related or unrelated individuals, even within a single population, sometimes along with more solitary individuals (Krützen *et al.* 2003; Owen 2003; Wiszniewski *et al.* 2012a). We do not have behavioral data to support the existence of male alliances in our studied population. Group sizes were larger than in the populations of Australia and Florida, making it difficult to follow the behavior of specific individuals (Chapter 3, Wells *et al.* 1987; Wiszniewski *et al.* 2009). No male alliances were recorded in other populations of the NEA, i.e. in the Moray Firth (Scotland, Wilson 1995) and the Sado estuary (Portugal, Augusto *et al.* 2011). In contrast to some populations, there was no segregation by sex, which may indicate lower female harassment by males (Fury *et al.* 2013).

Strongly associated individuals had similar ecology while individuals that never associated could present either dissimilar or similar ecology. Indeed, for individuals never associated, similar isotopic signatures could be obtained because of the consumption of the same prey in the same habitat, or different prey in distinct habitats having similar baseline stable isotope values. They could have dissimilar stable isotope signatures as a result of the consumption of different prey in the same habitat or the same prey in contrasting habitats. Further work investigating stable isotope values in potential prey of bottlenose dolphins is needed to better understand their ecology. Cooperative hunting has been observed in several populations (Gazda *et al.* 2005; Torres & Read 2009; Daura-Jorge *et al.* 2012), however we do not know which feeding techniques are used in Normandy. Large group sizes and the turbidity of the waters make it impossible to observe underwater behavior. Individuals sharing feeding strategies preferentially associate in other populations, e.g. individuals using sponges (Mann *et al.* 2012) and interacting or cooperating with fisheries (Ansmann *et al.* 2012a; Daura-Jorge *et al.* 2012; Pace *et al.* 2012). Given the stable isotope results, shared feeding ecology is likely a factor that led to preferential associations between individuals in the English Channel. As kinship does not drive associations, spending time with unrelated individuals might provide mutual benefits when foraging (Clutton-Brock 2009). It is however difficult to disentangle if dolphins associate because of similar foraging behavior, or if they show similar ecology as a result of transmission and learning from their associates (Daura-Jorge *et al.* 2012; Cantor & Whitehead 2013). Deviance explained by ecological similarity is low (approximately 5%). When provided, deviance values obtained in other studies ranged

from 17% to 31% (Mann *et al.* 2012; Carter *et al.* 2013). As statistical methods are not yet available for matrix data to enable the interaction of variables to be tested, the deviance explained by MRQAP is usually smaller than in standard linear regression. In addition, as individuals were sampled over two years, seasonal variations in stable isotope values, although minimal, could reduce the correlation between ecological homophily and association strength.

Other factors are likely to contribute to bottlenose dolphin social structure. Shared reproductive state could influence female associations (Möller & Harcourt 2008). Age, although difficult to monitor in dolphins, was a good predictor of associations in several fission-fusion species (Wey & Blumstein 2010; Hauver *et al.* 2013). Previous familiarity, in particular during the first years of life (Connor *et al.* 2000; Stanton *et al.* 2011) could influence associations in adulthood. For instance, dolphins are capable of long-term memory and individual recognition through individually specific vocal labelling (Bruck 2013; King & Janik 2013). Moreover, associating with familiar individuals was shown to confer fitness benefits (e.g. in fish and birds, Griffiths *et al.* 2004; Grabowska-Zhang *et al.* 2012). Personalities could also affect animal affiliative behavior (e.g. Croft *et al.* 2009; Aplin *et al.* 2013). Finally, predation is another major force that can influence social structure (see review in Krause & Ruxton 2002). In the Normano-Breton gulf, killer whales and shark species are not observed and no shark bites were ever recorded, which contrasted with Australian and North-West Atlantic inshore populations (Wells *et al.* 1987; Heithaus 2001). This lack of predation could have important evolutionary impact and might contribute to the absence of effect of relatedness on female social structure.

Non-social factors, such as habitat use, are increasingly included in social structure analyses to tease apart preferential associations and relationships resulting only from shared use of space (Frère *et al.* 2010b; Cantor *et al.* 2012; Carter *et al.* 2013). As this study included dolphins sighted in only at least five occasions, home ranges could not be included in the regression analysis. However, as dolphins are highly mobile, associations may reflect social preferences, at least to some degree, even in the case of overlapping ranges. In addition, shared use of space can be an indirect social factor by creating an opportunity for individuals to interact.

c) Influence of phylogeography on social structure

For some species, social structure can be strongly constrained by phylogenetics rather than being influenced by ecological selection (Di Fiore & Rendall 1994; Chapman & Rothman 2009). Coastal bottlenose dolphins in the North-East Atlantic (NEA) are genetically closer to the pelagic bottlenose dolphins of both the NEA and North-West Atlantic (NWA) than to coastal bottlenose dolphins in the NWA (Chapter 5). Moreover, recent genetic studies using mitochondrial DNA suggested that coastal populations were founded by the pelagic population more recently in the NEA than in the NWA (Chapter 5, Moura *et al.* 2013). Environment type is thought to have an influence on delphinids social structure. In a review of delphinids social structure, Möller (2011) predicted that females in inshore environments (estuaries or bays) will have associations of moderate strength with both kin and non kin although they will preferentially share stable associations with related females. In coastal open shorelines and pelagic environments, female associations should mainly be weak and not influenced by kinship. However, if resources are limited or the population is geographically isolated, associations might be moderate or strong (Möller 2011). Knowledge on pelagic bottlenose dolphin social structure is very limited. However, photo-identification studies showed low re-sighting rates of pelagic individuals around the Azores (Silva *et al.* 2008) and telemetry indicated long-distance movements in the NWA (Wells *et al.* 1999). In addition, other small pelagic delphinids have usually weak social structures that are not influenced by kinship (see review in Möller 2011). Coastal bottlenose dolphins in the English Channel might therefore have a social structure derived from pelagic Atlantic bottlenose dolphins rather than similar to the ones of inshore bottlenose dolphins in the NWA and Australia. In contrast to what is known for pelagic bottlenose dolphins (Silva *et al.* 2008), individuals are resident year-round in coastal waters of the English Channel (Chapter 3, stable isotope results of this chapter). Resource availability is a major factor driving marine top predator distribution and movements (Boyd *et al.* 1994; Fauchald & Erikstad 2002). For instance, for coastal bottlenose dolphins in California, the absence of evidence of site fidelity may be linked to the unpredictable and patchy distribution of prey (Defran & Weller 1999; Defran *et al.* 1999) and similar conclusions were drawn for transient pelagic bottlenose dolphins around the Azores (Silva *et al.* 2008). Ecological conditions might therefore be suitable to host a large population of dolphins in the English Channel. In addition, large group sizes might be

explained by prey predictability and availability (Chapter 3) but also phylogenetic constraints as pelagic groups are generally larger than coastal ones (Connor *et al.* 2000; Silva 2007).

d) Drivers of social structure and interest of combining approaches

The results show that ecology, individual foraging behavior and population structure history may have an influence on the social structure of coastal bottlenose dolphins. The absence of predation, resource availability and a recent founder event from the pelagic population probably played a role in shaping social structure characteristics specific to this population, i.e. large group sizes for resident coastal individuals and the absence of influence of relatedness. Suitable ecological conditions probably led to the residency of the individuals. This study highlights the importance to include phylogeography to better understand social organization, which is often ignored in cetacean studies (apart from a few studies such as the one of Beck *et al.* 2012). Similarly, killer whale social structure in the NEA is likely shaped by ecological conditions but phylogenetic inertia might also partially play a role (Beck *et al.* 2012). This work contributes to a growing number of studies showing that bottlenose dolphin societies, known to be fission-fusion, are highly variable within this form of social structure (Lusseau 2003; Wiszniewski *et al.* 2010b; Connor *et al.* 2011; Ansmann *et al.* 2012a; Daura-Jorge *et al.* 2012; Wiszniewski *et al.* 2012a). This might be explained by the wide range and contrasted type of habitats where the species occurs where ecological forces driving social structure can differ. The combination of approaches enabled us to get a better understanding of the structure of the population. A single genetic population was identified, whilst social structure and stable isotope analyses indicated three clusters. These results underlined the necessity to combine tools to assess fine-scale population structure, which is particularly important for conservation. We also showed that stable isotopes are useful to evaluate the influence of ecology on social structure and are particularly relevant in areas where foraging behavior of bottlenose dolphins cannot be monitored visually. This approach could be used for a wide range of cryptic or difficult to observe taxa. Further work, on stable isotopes in potential prey species could help to better understand foraging specializations within the population.

CHAPTER

5

HABITAT-DRIVEN POPULATION STRUCTURE OF BOTTLENOSE DOLPHINS IN THE NORTH-EAST ATLANTIC

1) Introduction

Despite no obvious physical barrier to gene flow and high movement capacities, intraspecific population differentiation in vertebrates can be high at large and small spatial scales (e.g. Natoli *et al.* 2004; Hoffman *et al.* 2005; Sacks *et al.* 2005). Environmental factors, in particular habitat characteristics and past climate changes, have been correlated with population divergence in fishes and mammals (e.g. Bernatchez 1997; Gaggiotti *et al.* 2009; Amaral *et al.* 2012b). The degree of connectivity between populations can also be influenced by an interaction between ecological conditions and behavioral traits. In fishes, natal homing (i.e. site fidelity to natal breeding ground) is suggested as an important factor shaping genetic differentiation among populations through local adaptation to a particular habitat that confers better fitness (e.g. Kawecki & Ebert 2004; Dionne *et al.* 2008). Similarly, despite high mobility, terrestrial carnivores (e.g. wolves and coyotes) can show cryptic population structure linked to individual preferential dispersal towards similar natal area habitats where they will find familiar prey resources (Sacks *et al.* 2005; Pilot *et al.* 2012). Resource specializations may also explain genetic differentiation of killer whales in the Pacific between sympatric fish and marine mammal eating ecotypes (Hoelzel *et al.* 1998a), and in the North-East Atlantic (NEA) among different fish eating populations (Foote *et al.* 2011). Social cohesion and learning of foraging techniques within the matrilineal pod is likely to promote philopatry (Hoelzel *et al.* 1998a).

Niche specializations between genetically different groups of individuals can result in the classification of ecotypes. The term "ecotype" was first defined in plants following common garden experiments (Turesson 1922a, b) and corresponded to ecological units that arise from genotypical responses to particular habitats. Groups of individuals in distinct environments can become differentiated, resulting in different ecotypes, if heritable variation is sufficient for natural selection to take place and if local adaptation is stronger than gene flow between groups (Begon *et al.* 2006). Since its first appearance, the definition of an ecotype has been controversial (see review in Lowry 2012). We used Lowry's (2012) ecotype definition in this study, i.e. groups of populations, which differ across the landscape by

genetics (e.g. allele frequencies differences) and ecological and/or physiological traits. Ecotype differentiation can be confirmed using common garden experiments for small animals like Dominican anoles (Thorpe *et al.* 2005). However, for large, highly-mobile mammals, these experiments would be impractical and ethically controversial. Molecular, ecological, distribution and behavioral studies are therefore needed. Killer whales in the North-East Pacific were classified in three ecotypes (resident, transient and offshore) from an in-depth knowledge of foraging behavior, genetics, ranging patterns and morphology (see review in de Bruyn *et al.* 2013). Coastal and pelagic bottlenose dolphin, *Tursiops truncatus*, ecotypes were distinguished through genetics, distribution, diet, and skull morphology in the North-West Atlantic (NWA) (Mead & Potter 1995; Hoelzel *et al.* 1998b) and in the Pacific (Walker 1981; Curry & Smith 1998; Perrin *et al.* 2011). The two bottlenose dolphin ecotypes form separate mitochondrial lineages in the NWA, with less genetic diversity in coastal populations. The situation is more complex in the Pacific Ocean and the North-East Atlantic (NEA) (Natoli *et al.* 2004; Tezanos-Pinto *et al.* 2009). In the Pacific, mitochondrial DNA (mtDNA) genetic differentiation between coastal and pelagic bottlenose dolphins is significant but there is no complete lineage sorting* (Segura *et al.* 2006). Tezanos-Pinto *et al.* (2009) suggested that ecotype differentiation in the NWA may not be representative of genetic structuring of bottlenose dolphins worldwide.

In the NEA, bottlenose dolphins are found in coastal waters where they form either discrete small resident groups of tens to hundreds of individuals (e.g. Berrow *et al.* 2012; Cheney *et al.* 2012) or more mobile groups (O'Brien *et al.* 2009). They are transient and/or resident in deep waters near offshore islands (Silva *et al.* 2008), the Gibraltar Strait (de Stephanis *et al.* 2008a) and pelagic waters in particular the shelf edge of the Bay of Biscay and Celtic Sea with abundance estimates of thousands of individuals (Hammond *et al.* 2009; Hammond *et al.* 2013). In the Mediterranean Sea, resident populations and mobile individuals were also reported (e.g. Gnone *et al.* 2011). There is a distributional hiatus in the NEA, i.e. resident coastal populations are mainly observed in shallow waters less than 40 m deep, while the sightings of large-scale surveys are mainly concentrated on the outer shelf, the shelf-edge (depths from 200 to 4000 m) and oceanic waters. There are also occasional sightings on the rest of the shelf (Certain *et al.* 2008, SAMM aerial campaigns 2011/2012, E. Pettex, pers. comm.; Hammond *et al.* 2013). Given this shallow coastal *vs.* deep pelagic habitat distribution, the existence of two distinct ecotypes could be possible. However, no

previous study attempted to delineate ecotypes in the NEA. Fine-scale genetic structure was reported locally in Ireland and the Iberian Peninsula where a potential differentiation between pelagic and coastal dolphins was suggested (Fernandez *et al.* 2011b; Mirimin *et al.* 2011). In contrast, despite high geographical distance, no differentiation was found between individuals sampled around the pelagic islands of Madeira and the Azores using a relatively small set of 10 microsatellites markers (Quérouil *et al.* 2007). The only large-scale genetic study (Natoli *et al.* 2005) correlated genetic breaks to oceanographic boundaries between Scotland and the NEA (using samples from South England to Gibraltar for the latter) and between West and East Mediterranean Sea. However, despite samples coming from Scotland to the Black Sea, this study was limited by small sample sizes (e.g. 35 samples for the NEA) and the relatively low number of microsatellites used (9). Our understanding of the bottlenose dolphin population structure is therefore extremely fragmented in the NEA. Determining population structure and delineating eventual bottlenose dolphin ecotypes in the NEA is essential for management as anthropogenic pressures can be extremely different in coastal and pelagic environments. The small size of resident coastal populations and the extinction of at least one genetically isolated population in an estuary (Humber Estuary, England) that has not been repopulated raised conservation concerns for the species in coastal waters (Nichols *et al.* 2007). Moreover, bottlenose dolphins are protected in Europe under the Habitats Directive where they are listed as a species whose conservation requires the designation of Special Areas of Conservation.

In this context, the aim of our study was to determine the population structure of bottlenose dolphins in the NEA. Thanks to a collaborative framework of organizations across Europe, we were able to gather a large sample size (i.e. 405 tissue samples) covering an unprecedentedly wide geographical area encompassing both coastal and pelagic waters. We used a combination of biopsy samples and samples from stranded animals and interpretation of data from strandings was enhanced by estimating, whenever possible, the most likely area of death of stranded individuals using a drift prediction model (Peltier *et al.* 2012). The most likely area of death is indeed more indicative of the individual living area than stranding location and the model is a promising and novel approach to improve the reliability of using stranded animals in genetic studies of marine megafauna. We also used a much larger set of independent loci (25 microsatellites and a 682 bp fragment of the mitochondrial control region) than previous studies. In addition, we worked with several clustering methods, which

is rarely done in marine mammal population structure studies. The identified populations were characterized in terms of genetic diversity, connectivity and effective population sizes. We placed our work in the broader phylogeographical context of the North Atlantic basin, which raised new hypotheses about the evolutionary history of bottlenose dolphins in this area. Finally, we discussed ecotype delineation, evolutionary scenarios, and ecological and behavioral processes driving the population structure of this highly mobile top predator.

2) Material and methods

a) Sample collection, DNA extraction and sexing

A total of 405 bottlenose dolphin samples were obtained from the NEA and the Mediterranean Sea (see study area in Figure 5.1). Samples were collected from free-ranging dolphins by skin biopsy sampling between 2003 and 2012 (N = 164 including the samples of the previous chapter) and from skin, muscle or kidney of stranded animals between 1990 and 2012 (N = 241). Tissue samples were either frozen or preserved in ethanol or DMSO. DNA was extracted using NucleoSpin Tissue kits (Macherey-Nagel) following the manufacturer's protocol.

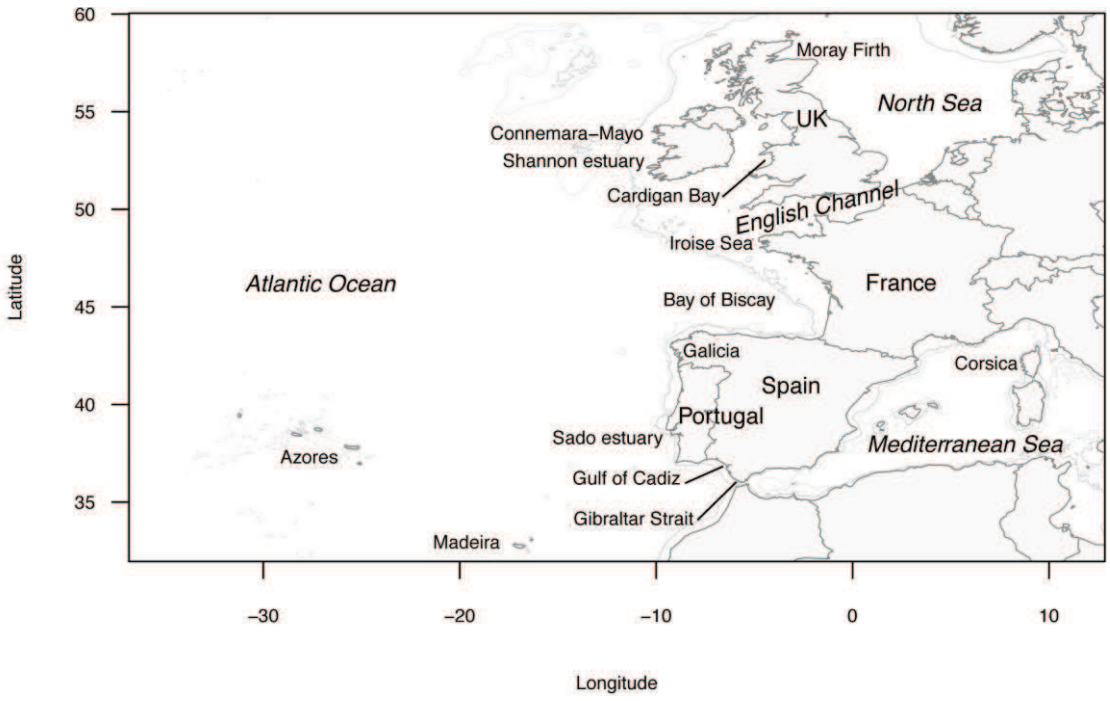


Figure 5.1. Map of the study area. -1000 and -200 m isobaths are plotted.

After checking for mitochondrial DNA (mtDNA) sequence quality and duplicates (i.e. individuals that were biopsy-sampled more than once), 381 samples (Figure 5.2) were kept in the analyses. 343 individuals had both mitochondrial and microsatellite data, 26 only mitochondrial data and 12 only microsatellites resulting in N = 355 for microsatellite and N = 369 for mitochondrial data analyses. Samples for which either mitochondrial or microsatellite data were missing came only from stranded individuals and the failure to obtain either mitochondrial or nuclear data is likely linked to decomposition state. Geographic origin was known for 173 samples (biopsy samples: N = 158; stranded animals that were previously photo-identified: N = 15), while 208 samples came from stranded animals of unknown origin. A drift prediction model which takes into account meteorological conditions (currents, winds and tides), the decomposition state of the carcasses and cetacean body parameters (thickness and floatability) was applied to stranded animals in the Bay of Biscay, English Channel and North Sea (the areas encompassed by the model), in order to estimate their most likely area of death (Appendix A5.1, Peltier *et al.* 2012). This could only be estimated when the decomposition state of the carcass was available (N = 66). The decomposition state is a proxy of the time after death in terms of intervals of days (Peltier *et al.* 2012). To estimate the most likely area of death, the centroid position of all the drift gps coordinates during the appropriate day interval was calculated for each individual using the geosphere package (Hijmans *et al.* 2012) in R 3.0.0. (R Core Team 2013). All maps were created using the marmap package (Pante & Simon-Bouhet 2013).

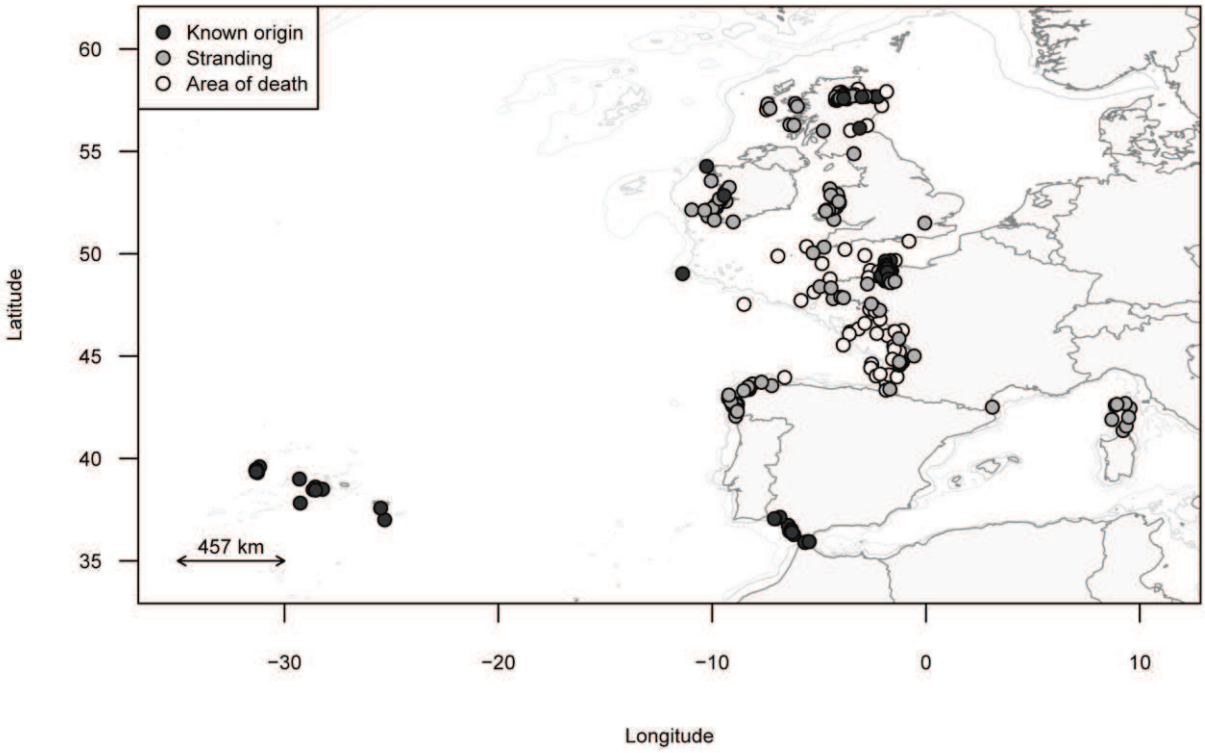


Figure 5.2. Sampling locations for individuals of known origin (biopsy samples and stranded individuals previously photo-identified), stranded animals (and their stranding locations) and areas of death (stranded animals for which it was possible to apply the drift prediction model). -1000 and -200 m isobaths are plotted.

The gender of the individuals was determined by amplification of the SRY plus ZFX/ZFY fragments as described in Rosel (2003) and/or visually during necropsy.

b) Microsatellite genotyping and validity

Samples were genotyped at the same 25 microsatellite loci as in Chapter 4 (see Chapter 2.1.d for general information on microsatellite markers, and Appendix A4.1 of the Chapter 4 for PCR, genotyping conditions and the characteristics of the microsatellite loci). To assess genotyping error rate, 28 individuals were randomly selected for re-amplification and scoring at all loci. 13 duplicates were also included in error rate calculation. 11.55% of the dataset was therefore reprocessed. Individuals were kept in the analyses when at least 12 loci were successfully amplified (N = 355) resulting in 1.84% of missing values in the whole

dataset. Each microsatellite locus was checked for null alleles and scoring errors using Microchecker 2.2.3 (Van Oosterhout *et al.* 2004). Departures from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium were tested using 10 000 iterations in GENEPOP web version 4.2 (Raymond & Rousset 1995). Tests were conducted for the whole dataset and for each population identified by the clustering methods. Significance levels were corrected for multiple comparisons using the sequential Bonferroni technique for this test and for all multiple comparisons of the study (Holm 1979).

c) Mitochondrial DNA sequencing

A 682 base-pair (bp) portion of the mitochondrial control region was amplified using primers Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') (Baker *et al.* 1998) and Dlp8G (5'-GGAGTACTATGTCCTGTAACCA-3') (as reported in Dalebout *et al.* 2005). PCR conditions are given in Appendix A4.4 of the previous chapter and the general characteristics of mitochondrial markers are described in Chapter 2.1.d. Consensus sequences were generated and checked for ambiguities with Sequencher 5.0 Demo (Gene Codes Corporation) and manually edited with BioEdit (Hall 1999). Unique haplotypes were identified using DNAsp (Rozas & Rozas 1999).

d) Population structure

We used three clustering methods to determine the most likely number of populations and assign individuals to these: a multivariate method, the Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.* 2010), and two Bayesian methods implemented in STRUCTURE (Pritchard *et al.* 2000) and TESS (Durand *et al.* 2009b). We used these different approaches to ensure that our results were reliable (Guillot *et al.* 2009). These methods are summarized in Chapter 4.2.d and detailed in Chapter 2.2.b. Parameter values and steps are given here again as there are slight changes.

DAPC, which is efficient at detecting hierarchical structure, was performed using the package adegenet (Jombart 2008) in R 3.0.0 (see Chapter 4.2.d for details). Membership

probabilities were calculated for each individual and each individual was assigned to a cluster using its maximum membership probability.

In STRUCTURE, the admixture models with correlated and uncorrelated allele frequencies were used, without indicating any *a priori* information on the origin of the samples. Ten independent runs for *K* values set from 1 to 10 were performed using a burnin-period of 50 000 iterations followed by 300 000 Markov Chain Monte Carlo (MCMC) steps. The most likely number of clusters was chosen by calculating ΔK (Evanno *et al.* 2005), which is the second order rate of change of the mean loglikelihood of the data (Ln$P(D)$) between successive *K* values in STRUCTURE Harvester v.0.5 (Earl & Vonholdt 2012). As this method cannot identify $K = 1$, we confirmed the results by plotting Ln$P(D)$ (Pritchard *et al.* 2000), examining individual membership proportion plots and consistency across runs. The Evanno method can reveal hierarchical structure by detecting the upper level of genetic differentiation (Evanno *et al.* 2005), therefore STRUCTURE was re-run in each of the identified clusters. When *K* was defined, the run with the highest Ln$P(D)$ value was selected and individuals were assigned to clusters based on maximum membership proportions[3].

The conditional auto-regressive (CAR) admixture model was run in TESS using a burnin of 20 000 steps followed by 120 000 MCMC steps. The number of clusters (*K*) to test was set from 2 to 10, with 10 replicate runs for each *K*. The spatial interaction parameter was set to 0.6 and the degree of trend to linear (which are the default parameters). To exclude land masses from the analysis, 9 dummy points were added along French and Spanish coasts (Durand *et al.* 2009a). The most likely number of clusters was selected by plotting Deviance Information Criterion (DIC) values against *K* and by examining plots of individual membership proportions. Consistency of the runs was checked. When *K* was defined, the run with the lowest DIC was used and individuals were assigned to clusters based on maximum membership proportions.

As results were highly consistent between analyses in terms of the most likely number of clusters and individual assignments (which were identical for 93.53% of individuals across the three methods), the method that uses both multi-locus genetic data and spatial coordinates

[3] We use "membership proportions" to refer to the percentages of the genome of an individual that come from each population (i.e. admixture proportions, see Chapter 2.2.b for details). For vocabulary simplification, we will use individual assignment to populations when referring to the clustering results instead of the assignment of individual's genomes.

(i.e. TESS) was used to divide the dataset into populations for the following analyses (see description of the populations in the population structure result section).

As the inclusion of closely related individuals could impact population structure analyses, the Queller & Goodnight (Queller & Goodnight 1989) relatedness coefficient (*R*) was calculated using KINGROUP v.2 (Konovalov *et al.* 2004) within each population identified by TESS. TESS was then re-run by removing one individual from each pair of individuals showing a relatedness coefficient superior or equal to 0.45 as in Rosel *et al.* (2009).

Sex-biased dispersal was tested in FSTAT 2.9.3 by comparing sex-specific assignment indices, relatedness, F_{ST} and F_{IS} values using 10 000 permutations (Goudet *et al.* 2002). The test was performed on the whole dataset using the populations identified by TESS and at the different levels of the hierarchical structure. Only adults were included in the test (biopsy samples were only collected from adults, and for stranded animals, we kept only individuals with a minimum total length of 250 cm, i.e. an arbitrary threshold for which we considered that individuals were physically mature, N = 292 individuals).

e) Nuclear genetic differentiation and diversity

To characterize the level of genetic differentiation among the clusters identified by TESS, pairwise F_{ST} were estimated between populations using Arlequin 3.5.1.3 (Michalakis & Excoffier 1996). The level of significance was assessed using 10 000 permutations. The analyses were also performed with the dataset excluding closely related individuals. For each identified population, mean number of alleles (NA) and allelic richness* (AR) were calculated in FSTAT (Goudet 1995). Inbreeding coefficient (F_{IS}), observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated in Arlequin. Convert (Glaubitz 2004) was used to identify private alleles*. Diversity indices were also calculated per locus. Mean AR and *Ho* were compared between pairs of populations using a Wilcoxon paired-sample test.

f) Mitochondrial DNA differentiation and diversity

A haplotype network was constructed to determine genealogical relationships using median-joining and maximum-parsimony algorithms implemented in Network 4.6.0.0 (Bandelt *et al.* 1999). Sequences were clustered according to the populations identified by TESS. Number of haplotypes (NH), number of polymorphic sites (S), haplotypic diversity* (h) and nucleotide diversity* (π) were determined for each population in Arlequin. jModeltest 2.1.3 was used to determine the most accurate model of substitution using the Bayesian Information Criterion (BIC, Guindon & Gascuel 2003). Pairwise genetic differentiation was estimated between populations in Arlequin using F_{ST} and Φ_{ST}. For Φ_{ST}, the Tamura and Nei (1993) model of substitution was chosen as it is the closest model to the HKY + I model, selected by jModeltest. Significance levels were tested using 10 000 permutations.

Sequences from this study were placed in the phylogeographical context of the North Atlantic basin. Haplotypes from the NWA, and additional sequences from the Azores and Madeira were obtained from GENBANK (Appendix A5.2). A haplotype network was constructed as described above using a 324 bp consensus length for unique haplotypes.

g) Recent migration rates

Recent and asymmetric migration rates (within the last few generations) among populations identified by TESS were estimated using the Bayesian method implemented in BayesAss (Wilson & Rannala 2003) on microsatellite data (see Appendix A5.3 for the settings).

h) Effective population sizes

We used two methods for estimating contemporary effective population sizes (N_e) for each population identified by TESS: a method that uses linkage disequilibrium in LDNe (Waples & Do 2008) and an Approximate Bayesian Computation method implemented in ONeSAMP (Tallmon *et al.* 2008). In LDNe, alleles frequencies less than 0.02 (P_{crit}) were excluded from the analyses to avoid bias caused by rare alleles but still get a high precision

(Waples & Do 2010). In ONeSAMP, N_e priors were set from 2 to 500 and from 2 to 10 000 for the expected small and large populations, respectively. Influences of priors on the estimates were tested for the two coastal populations, using priors from 4 to 1000 and from 2 to 200. Our dataset included multiple cohorts and age classes, which will bias N_e estimates downwards. For instance, a 10-15% downward bias in N_e estimates was observed in a study using mature bottlenose dolphins and a P_{crit} of 0.02 in LDNe (Robin Waples, personal communication). We therefore applied a bias correction of 15% to our results for both LDNe and ONeSAMP ($N_e c$).

3) Results

a) Microsatellite validity

The genotyping error rate was 0.0097 (i.e. 10 incorrect genotypes / 1025 genotypes reprocessed). The error rate for stranded individuals, which were fresh to moderately decomposed (0.013, i.e. 7 incorrect genotypes / 525 genotypes reprocessed), was twice as large as the error rate for live individuals (0.006, i.e. 3 incorrect genotypes / 500 genotypes reprocessed). Significant departure from HWE was detected for the majority of the loci when considering the whole dataset as a single population. However, this was the result of Wahlund effects* as no significant departure was found when dividing the dataset into the populations identified by TESS, except for loci MK9 and EV37 in one population each (Appendix A5.4). As deviation was significant in only one population and results with and without these two loci were essentially the same (number of clusters and individual assignments), only results including MK9 and EV37 are reported. Linkage disequilibrium was significant for 0.50% of the pairwise comparisons and when significant, it was not detected across all populations, and was therefore considered negligible.

b) Drift prediction model

The drift prediction model indicated that individuals were likely to have died in coastal waters in the North-Sea and the English Channel and from coastal to the outer shelf-edge waters in the Bay of Biscay (Appendix A5.1).

c) Population structure

Four populations and a pattern of hierarchical structure were identified using DAPC (Figure 5.3A). The first component separated two clusters that were further divided into two clusters by the second component (BIC plot in Appendix A5.5). The most likely number of clusters identified using STRUCTURE and the Evanno method was two (Figure 5.3Ba, Evanno plot in Appendix A5.6a), using both the correlated and uncorrelated allele frequency models. The majority of individuals (98%) were strongly assigned to one of the clusters (membership proportions $Q > 0.80$). As the DAPC indicated a hierarchical structure, STRUCTURE was re-run inside each of the two clusters. A further division was found within each of the two clusters (Figures 5.3Bb and 5.3Bc, Evanno plots in Appendix A5.6b and A.5.6c) with strong assignments for most individuals (96%).

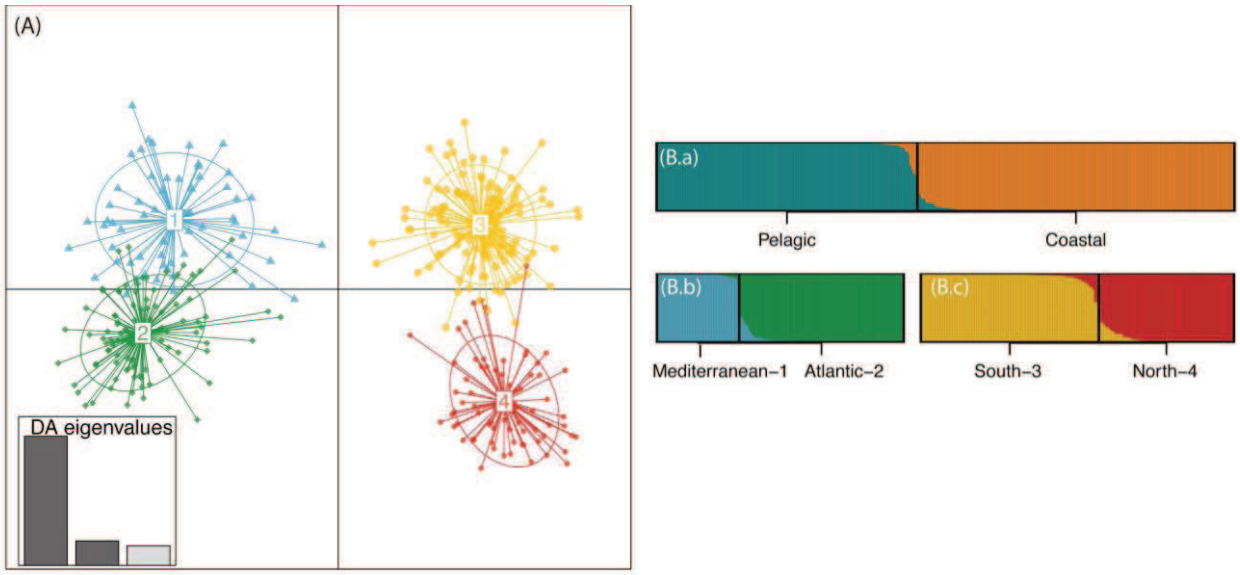


Figure 5.3. (A) DAPC scatterplot showing the first two principal components for K = 4 (Mediterranean = 1, Atlantic = 2, South = 3, North = 4). (B) Bayesian membership proportions of individual bottlenose dolphins inferred using STRUCTURE. Each vertical column corresponds to one individual, with the colors representing the membership proportions to each of the two clusters. Dolphins were sorted using their maximum membership proportions. The black vertical lines delimit the inferred populations. (a) Barplot for the highest level of genetic structuring between pelagic and coastal dolphins. Barplots for the second level of genetic structuring between (b) Mediterranean and Atlantic pelagic dolphins, and (c) South and North coastal dolphins.

Finally, TESS detected four populations (Figure 5.4, DIC plot in Appendix A5.7), with 93% of individuals strongly assigned (Q > 0.80). Assignments were highly consistent among the methods with 93.5% of the individuals assigned to the same cluster across the three methods. Moreover, comparisons of TESS barplot (K = 4) and STRUCTURE barplot for K = 4 also indicated almost identical results for individual assignments (data not shown). Therefore, we considered that the population structure signal was strong and not linked to analytical artifacts.

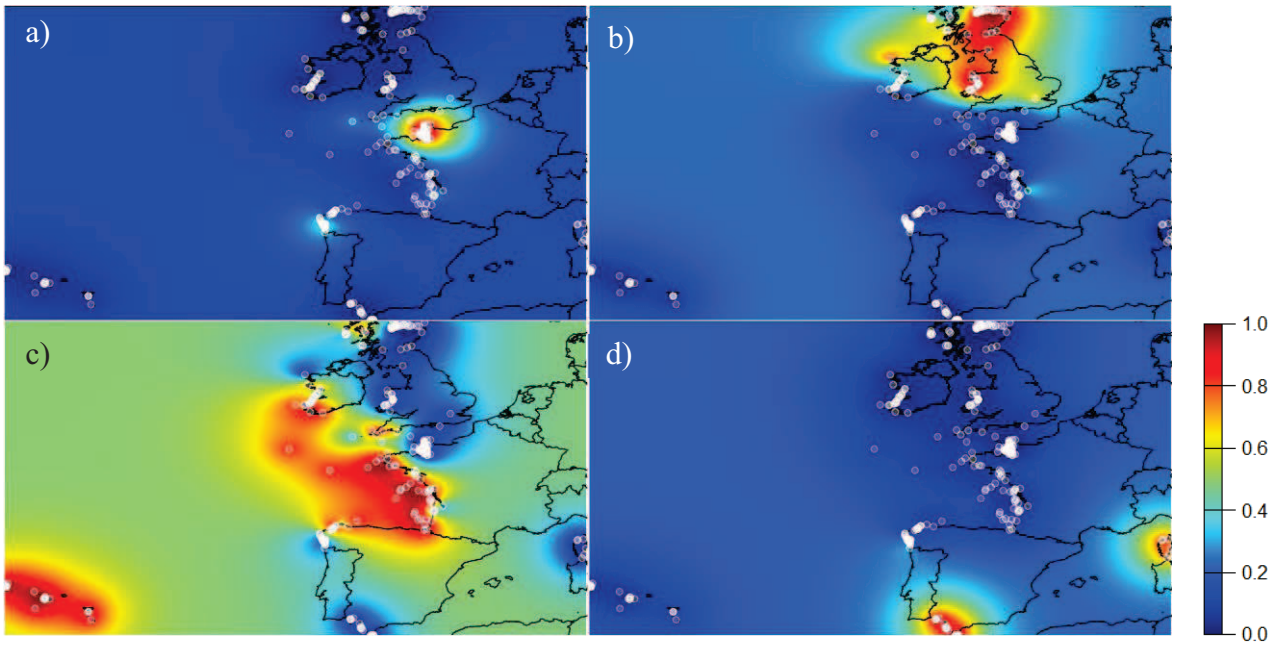


Figure 5.4. Map of individual membership proportions per population identified by TESS. The color scale bar indicates the membership proportions, (a) Coastal South, (b) Coastal North, (c), Pelagic Atlantic (d) Pelagic Mediterranean.

The first population identified by TESS (N = 119) was composed of individuals that were biopsy sampled or that stranded in the English Channel (France), three resident individuals that stranded in the Bay of Biscay (France) and stranded animals in South Galicia (Spain). The second cluster (N = 77) was composed of individuals biopsy sampled or stranded in Ireland, England or Scotland (including 10 previously photo-identified resident dolphins for the latter). These two clusters grouped together in the first level of differentiation identified by STRUCTURE and DAPC. These individuals were biopsy sampled in shallow and coastal waters (less than 20 m deep), or stranded in areas near resident populations (i.e. English Channel, Cardigan Bay (Wales, United Kingdom), Moray Firth (Scotland), South Galicia rias (Spain)) and included dolphins previously photo-identified. Moreover, for these populations, the most likely area of death of individuals for which it was possible to apply the drift prediction model indicated that they came only from coastal and shallow waters. These two populations were therefore composed by coastal dolphins, and named "Coastal South" (English Channel, Arcachon estuary and South Galicia resident groups) and "Coastal North" (United Kingdom and Ireland resident or mobile coastal groups) populations. Individuals biopsy sampled in pelagic waters of the NEA (including the Azores archipelago) and stranded animals along the west coasts of Europe formed a third population (N = 107). According to

the drift prediction model, individuals were likely to have died from coastal waters to the shelf edge. The last population (N = 52) was composed of individuals biopsy sampled in the Gulf of Cadiz and the deep waters of the Gibraltar Strait and by individuals stranded in Corsica. These two populations grouped together in the upper level of structure revealed by STRUCTURE and the DAPC. As the biopsied dolphins in this group were sampled in deep waters (> 200 m) of the Azores, the NEA and the Gibraltar Strait, these two populations were composed of pelagic individuals and named "Pelagic Atlantic" and "Pelagic Mediterranean" populations.

The removal of one individual per pair of closely related individuals (25, 21 and 1 individuals were removed from the Coastal South, Coastal North and Pelagic Mediterranean populations, respectively) did not change the inferred population structure.

Gender was determined for 370 individuals (153 females, 217 males). No significant sex-biased dispersal was found for any of the tested indices (all $P > 0.05$) either among the four populations or between each of two main groups (coastal and pelagic). We had reasonable numbers of males and females in each group for the 292 adults included in the sex-biased dispersal test (Coastal North = 23 females + 26 males, Coastal South = 39 females + 70 males, Pelagic Atlantic = 32 females + 56 males and Pelagic Mediterranean = 20 females and 26 males).

A total of 55 mitochondrial DNA (mtDNA) haplotypes were identified in the NEA dataset (including 53 haplotypes for individuals that were also genotyped for microsatellites, see Appendix A5.8 for the table of polymorphic sites). The median joining-network (Figure 5.5) indicated that the majority of individuals in the coastal group shared haplotypes forming a lineage separated by 12 base pairs (bp) from the lineage including most haplotypes found in the pelagic group. Only two haplotypes were shared between the coastal and the pelagic group. Some haplotypes within the pelagic group were highly divergent, with 49 bp separating the two most distant haplotypes.

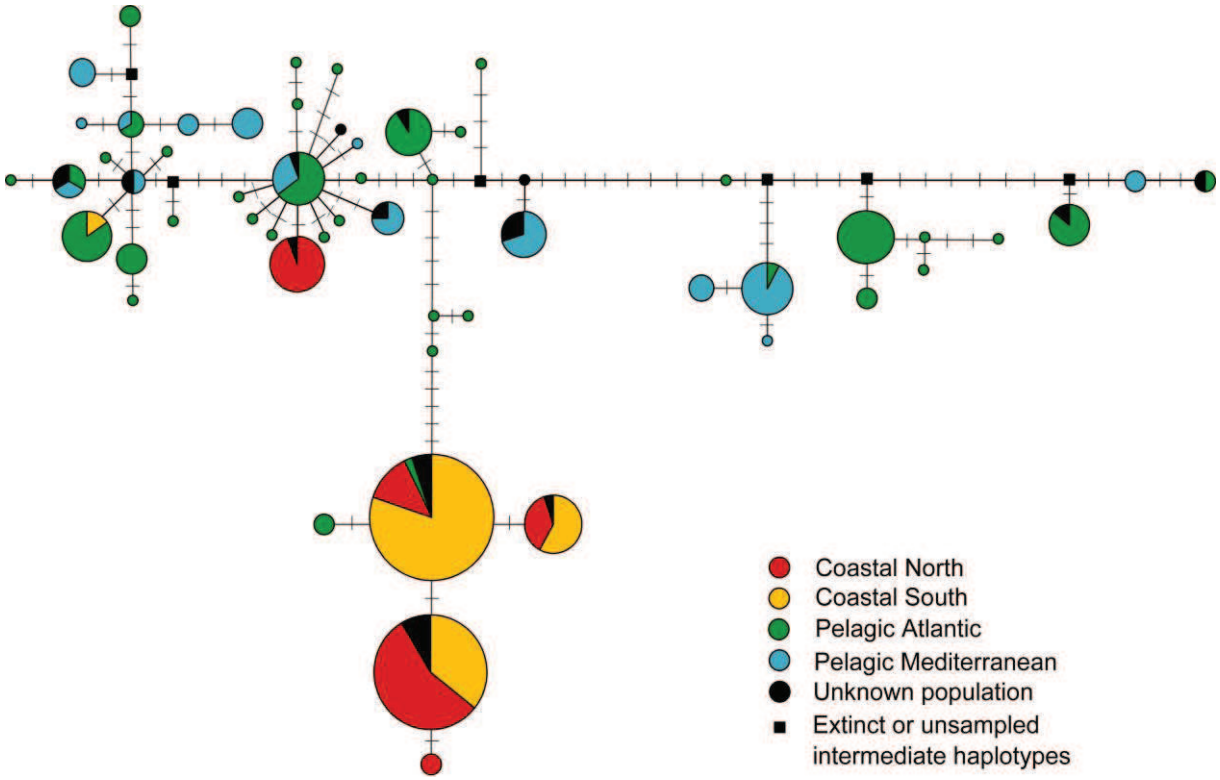


Figure 5.5. Median-joining network of mtDNA control region haplotypes found in bottlenose dolphins from the North-East Atlantic. Each circle represents a unique haplotype colored in proportion to the number of individuals from the populations inferred by TESS that share the haplotype (individuals for which the population could not be inferred by microsatellite data are shaded in black). Size of circles is proportional to haplotype frequencies. Black squares indicate either extinct or unsampled intermediate haplotypes. Black dashes indicate mutation steps between haplotypes.

When using only 324 bp sequences to include haplotypes from other studies, the number of haplotypes was reduced from 6 to 4 for NEA coastal dolphins, and from 49 to 38 for NEA pelagic dolphins (Figure 5.6). Haplotypes of the NWA were classified as coastal or pelagic following designation used in previous studies (listed in Appendix A5.2 and Patricia Rosel, personal communication). Coastal haplotypes from the NWA formed a completely separate lineage. Haplotypes from NEA and NWA pelagic individuals, from the Azores and Madeira, and from NEA coastal individuals were clustered together in the network. Eighteen haplotypes were shared between NWA pelagic and NEA pelagic, NEA coastal or Azores and Madeira dolphins.

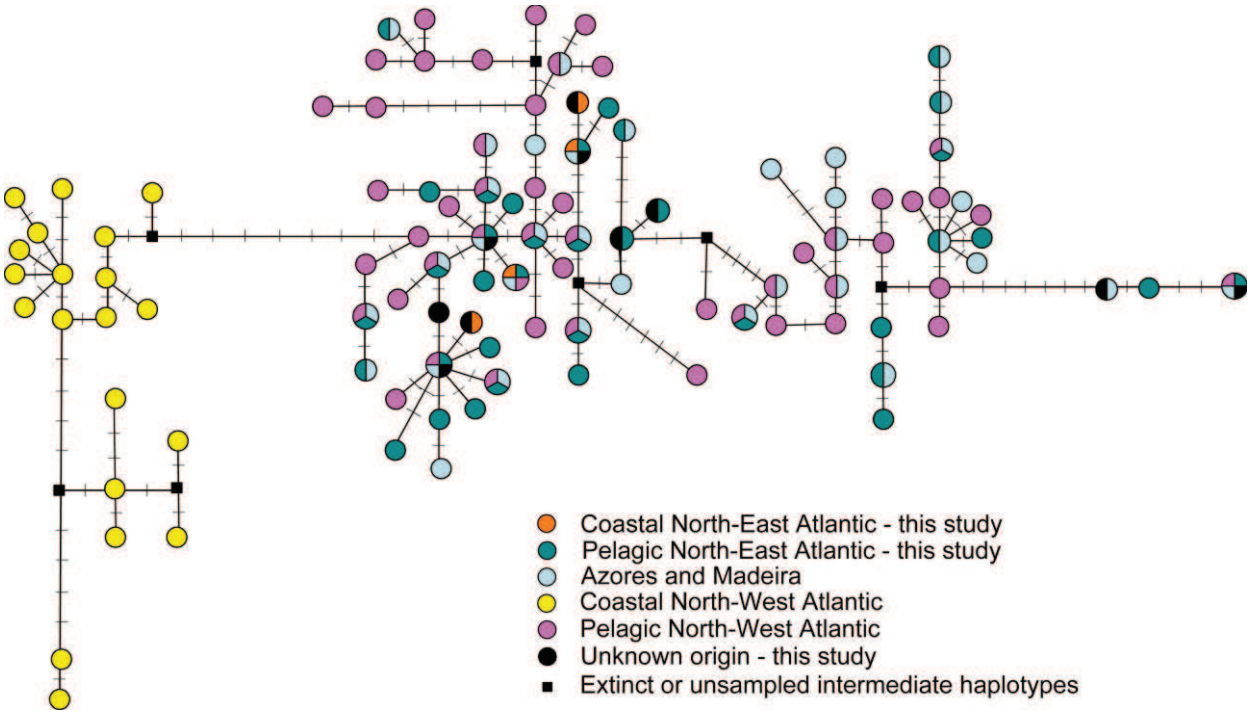


Figure 5.6. Median-joining network of mtDNA control region haplotypes found in bottlenose dolphins from the North Atlantic. Each circle represents a unique haplotype colored according to the population where it was found. The haplotype frequencies were not taken into account. The two pelagic and coastal populations of this study were grouped. Black squares indicate either extinct or unsampled intermediate haplotypes. Black dashes indicate intermediate mutation steps between haplotypes.

d) Genetic differentiation and genetic diversity in the NEA

All nuclear F_{ST}, mtDNA F_{ST} and Φ_{ST} pairwise comparisons were significant, with the highest level of differentiation found when comparing pelagic and coastal populations (Tables 5.1 and 5.2). Comparisons of the two coastal populations also had a high mtDNA F_{ST} value. As identical results were obtained when excluding closely related dolphins, they were kept in the analyses.

Table 5.1. Pairwise microsatellite F_{ST} between populations.

Population	Coastal South	Coastal North	Pelagic Atlantic	Pelagic Mediterranean
Coastal South (N = 119)	-	0.057**	0.133**	0.118**
Coastal North (N = 77)		-	0.149**	0.157**
Pelagic Atlantic (N = 107)			-	0.043**
Pelagic Mediterranean (N = 52)				-

** $P < 0.01$ after sequential Bonferroni correction.

Table 5.2. Pairwise mitochondrial F_{ST} (above diagonal) and Φ_{ST} (below diagonal) between populations.

Population	Coastal South	Coastal North	Pelagic Atlantic	Pelagic Mediterranean
Coastal South (N = 115)	-	0.252**	0.279**	0.326**
Coastal North (N = 76)	0.233**	-	0.195**	0.221**
Pelagic Atlantic (N = 101)	0.541**	0.349**	-	0.071**
Pelagic Mediterranean (N = 51)	0.671**	0.445**	0.056**	-

** $P < 0.01$ after sequential Bonferroni correction.

Mitochondrial genetic diversity was higher in pelagic populations than in coastal populations (Table 5.3). Despite similar sample sizes, the number of haplotypes in the coastal populations was considerably lower than in pelagic populations, with the majority of coastal individuals sharing two haplotypes and with no evidence of most common pelagic haplotypes (see Appendix A5.9 for haplotype frequencies by population).

Nuclear genetic diversity (Allele Richness* (AR) and Observed Heterozygosity (*Ho*)) was significantly lower in coastal than in pelagic clusters (Wilcoxon test, $P < 0.01$, Table 5.3, Appendix A5.4 for values per loci per populations). All pairwise comparisons were significant except for the AR, which was not significantly different between the two coastal clusters. Lower numbers of private alleles were identified in coastal populations than in pelagic populations (Table 5.3). A significant heterozygote deficiency was detected in the Coastal North population (Table 5.3), which was likely due to the inclusion of closely related individuals since F_{IS} was non-significant when they were removed ($F_{IS} = 0.029$, $P = 0.119$).

Table 5.3. Mitochondrial and nuclear diversities for each population inferred by TESS.

	Mitochondrial					Microsatellites						
Populations	N	No hapl.	S	*h*	*Π*	N	F_{IS}	*P*	*Ho*	*He*	NA	AR	PA
Coastal South	115	4	12	0.499 (0.044)	0.001 (0.001)	119	0.012	0.240	0.582 (0.180)	0.596 (0.172)	6.3 (2.8)	5.8 (2.6)	2
Coastal North	76	5	13	0.667 (0.042)	0.006 (0.003)	77	0.062	0.002	0.486 (0.180)	0.541 (0.191)	5.8 (2.4)	5.3 (2.2)	2
Pelagic Atlantic	101	38	41	0.929 (0.013)	0.014 (0.007)	107	0.008	0.236	0.734 (0.131)	0.770 (0.131)	9.8 (3.9)	9.0 (3.3)	48
Pelagic Mediterranean	51	15	28	0.902 (0.022)	0.013 (0.007)	52	0.018	0.154	0.700 (0.158)	0.726 (0.140)	7.8 (3.4)	7.8 (3.4)	8
Overall *	369	55	46	0.883 (0.011)	0.012 (0.006)	355	0.103	0.000	0.631 (0.139)	0.715 (0.142)	10.8 (5.2)	8.7 (3.7)	-

N = number of individuals, No hapl. = number of haplotypes, S = number of polymorphic sites, *h* = haplotypic diversity, π = nucleotide diversity, F_{IS} = inbreeding coefficient, *P* = F_{IS} *P*-value, *Ho* = observed Heterozygosity, *He* = expected Heterozygosity, NA = mean Number of Alleles, AR = mean Allelic Richness, PA = total number of Private Alleles, SD in parenthesis when appropriate. *26 individuals that were not included in microsatellites analyses (due to amplification issues), and thus were not assigned to any population, were included in the overall values of mtDNA diversities. 12 individuals were successfully amplified for microsatellite markers but not for mtDNA.

e) Recent migration rates

Estimates were highly consistent between runs, therefore results for a randomly chosen run were selected (Table 5.4). Estimates of recent migrations rates were low among all clusters: 1.1% per generation at most, and with 95% confidence intervals that included 0 (Table 5.4).

Table 5.4. Mean (and 95% CI) recent migration rates inferred using BayesAss. The migration rate is the proportion of individuals in a population that immigrated from a source population per generation.

To From	Coastal South	Coastal North	Pelagic Atlantic	Pelagic Mediterranean
Coastal South	0.990 (0.979-1.000)	0.004 (0.000-0.012)	0.003 (0.000-0.008)	0.003 (0.000-0.009)
Coastal North	0.008 (0.000-0.021)	0.984 (0.967-1.000)	0.004 (0.000-0.012)	0.004 (0.000-0.012)
Pelagic Atlantic	0.004 (0.000-0.010)	0.003 (0.000-0.009)	0.983 (0.956-1.000)	0.011 (0.000-0.026)
Pelagic Mediteranean	0.010 (0.000-0.026)	0.009 (0.000-0.024)	0.009 (0.000-0.024)	0.973 (0.947-0.999)

The diagonal values represent the proportion of nonimmigrants in a population.

f) Effective population sizes

The two methods produced roughly similar contemporary effective size ($N_e c$) estimates, with considerably lower estimates for coastal populations than for pelagic populations (Table 5.5). Using different priors for coastal populations in ONeSAMP, $N_e c$ estimates varied only slightly. Despite months of computation, pelagic population $N_e c$ estimates using ONeSAMP never converged.

Table 5.5. Contemporary effective population sizes corrected for overlapping generations ($N_e c$) estimated using LDNe and ONeSAMP

	LDNe	ONeSAMP
Coastal South	64 (56 - 74)	77 (62 - 108)
Coastal North	32 (28 - 37)	46 (36 - 62)
Pelagic Atlantic	7748 (1333 – infinite)	Endless run
Pelagic Mediteranean	231 (168 – 360)	Endless run

4) Discussion

a) Hierarchical structure

Bottlenose dolphins were hierarchically structured in the NEA. The strongest level of genetic differentiation was found between coastal and pelagic dolphins both with microsatellite and mtDNA markers. The NEA haplotype network indicated two separate mitochondrial lineages with no complete lineage sorting between coastal and pelagic dolphins. Shared haplotypes indicated possible migration, incomplete lineage sorting or introgression. As in the NWA (Natoli *et al.* 2004), genetic diversities were higher in pelagic than in coastal populations. Significant genetic structure was found within each of the two groups. Migration rates between populations were low (about 1% per generation or less). In the coastal group, individuals sampled in the UK and Ireland (Coastal North) formed one population. Eight dolphins were reported moving between east and west Scotland and between Scotland and Ireland coastal groups through photo-identification (Robinson *et al.* 2012), which suggests that these wide-ranging individuals may maintain genetic connectivity between resident groups. This population was differentiated from neighboring English Channel dolphins and more distant Galician individuals. However, several resident coastal groups (e.g. Shannon estuary, Ireland; Iroise Sea, France, Sado Estuary, Portugal) were not sampled. Moreover, the Shannon population is genetically isolated from other inshore dolphins in Ireland (Mirimin *et al.* 2011). Thus, more structuring is expected in coastal

waters. In the pelagic group, individuals from the NEA were separated from individuals sampled in the gulf of Cadiz, Gibraltar Strait and Mediterranean Sea. Individuals sampled in the Azores clustered with 88 individuals from the rest of the NEA, which can be surprising given the large distance between the Azores and the shelf edge. Deep waters (> 200 m) are found very close to shore for this archipelago indicating that bottlenose dolphins inhabit oceanic environments. Photo-identification work indicated that resident individuals represented less than 5% of individuals found in the Azores, the majority of the individuals being transients or migrants (Silva *et al.* 2008). This could explain the lack of structure found in Quérouil *et al.* (2007) and our study, which contrasted with other oceanic archipelagos where genetic structure was found, like in Hawaii, where shallow water areas are larger and high site fidelity has been reported (Martien *et al.* 2011). Individuals of the Mediterranean Sea were considered as coastal in previous studies (Natoli *et al.* 2004; Natoli *et al.* 2005), which contrasted with their high genetic diversity and with our results indicating that they were pelagic. Some coastal groups are resident but movements were reported between Corsica and France (Gnone *et al.* 2011), indicating that individuals crossed pelagic waters. The pelagic habitat use was confirmed by aerial surveys conducted during winter where bottlenose dolphins were mainly sighted in deep-water (> 200 m) areas (SAMM, 2011/2012, E. Pettex, pers. comm.). We could however not exclude further population structuring within this area as we had a limited sample size and only samples from stranded individual for Corsica. Biopsy sampling of coastal and pelagic groups is therefore needed to assess Mediterranean Sea bottlenose dolphin population structure.

To our knowledge, this is the first time that the structure and connectivity between and within pelagic and coastal bottlenose dolphin populations was investigated in the NEA. Three clustering methods relying on different assumptions produced extremely consistent results. We therefore concluded that the genetic signal is strong and inferences reliable. We emphasize that using different methods is particularly important when working on highly mobile animals for which geographical barriers are not obvious. It is still rarely done in marine mammal studies. In our case, the landscape genetic method was efficient at detecting and geographically delineating four populations. However, marine mammal studies using a landscape genetics approach are still scarce (but see Fontaine *et al.* 2007; Möller *et al.* 2011). Our study shed light on global patterns of population structure of bottlenose dolphins in the NEA. However, finer-scale population structure could exist within the identified populations,

as Bayesian clustering methods have been shown to be inefficient at detecting structure when differentiation levels are below F_{ST} of 0.02 (Latch *et al.* 2006; Chen *et al.* 2007). This work could therefore be the basis for more localized studies inferring finer-scale population structure.

Although sampling stranded animals is a cost effective method, we acknowledge that not all animals dying at sea are likely to strand (see review in Peltier *et al.* 2012), which confers uncertainty about the representativeness of these samples. The use of the most likely area of death (Peltier *et al.* 2012) for part of the stranded individuals shed light on their origin, which was consistent with the genetic results separating coastal and pelagic bottlenose dolphins. Unfortunately, meteorological data were not available for the whole area, making it impossible to apply the model for the complete dataset. In addition, the most likely area of death does not necessarily correspond to living areas in particular if sick or weakened animals move to another area to die (e.g. closer to shore). Despite these caveats, the likely position of death was more indicative of the individual living area than stranding position. Moreover, Peltier *et al.* (2012) drift experiments with tagged individuals indicated a high precision of the model: 27.1 ± 24.5 km (mean distance between the observed stranding positions of the tagged animals and the positions predicted by the model). It is therefore a promising tool for the use of stranded dolphins in genetic studies, which has recently been questioned (Bilgmann *et al.* 2011).

b) Possible drivers of population structure

A complex interaction between historic environmental processes and contemporary ecological and behavioral factors is likely to drive social cetacean population structure (Möller 2011; Amaral *et al.* 2012a; Amaral *et al.* 2012b).

For bottlenose dolphins in the NEA, given the topology of the haplotype network, a single founding event of the coastal populations from the pelagic population could be a possible evolutionary scenario. This hypothesis is supported by the low genetic diversities and small effective population sizes of coastal populations. Founder events often involve few individuals, which leads to a loss of genetic diversity due to genetic drift. A similar scenario is suggested for NWA bottlenose dolphins (Hoelzel *et al.* 1998b; Natoli *et al.* 2004). When

placing our samples in the Atlantic basin context, the NEA coastal haplotypes were more closely related to the NWA pelagic haplotypes than to the NWA coastal haplotypes. The pelagic population is possibly undifferentiated in the North Atlantic (Quérouil *et al.* 2007) although this needs to be confirmed using a larger sampling size and nuclear markers. Founder events might therefore have occurred independently from this wide-ranging pelagic population when suitable coastal habitats were released during interglacial periods (Natoli *et al.* 2004) on the two sides of the Atlantic basin, and more recently in the NEA than in the NWA. These hypotheses should be tested using coalescent approaches. Nevertheless, our work indicated that evolutionary history of bottlenose dolphins may differ among oceanic regions. In Chapter 6, we use Approximate Bayesian Computation demographic analyses to estimate divergence times between ecotypes in the NEA.

Genetically identified coastal bottlenose dolphins were only biopsy-sampled in shallow waters whereas genetically identified pelagic individuals were sampled in deep waters. This supports a habitat-driven population structure in bottlenose dolphins. Although sex-biased dispersal methods are known to have low power (Goudet *et al.* 2002) and thus caution should be taken when interpreting the results, we showed that both males and females were philopatric as found in several other bottlenose dolphin populations (see review in Möller 2011). This situation contrasted with the mammalian mating system where females tend to be philopatric as their reproductive success is mainly limited by food resources, while males tend to disperse as their reproductive success is constrained by access to mates (Emlen & Oring 1977; Greenwood 1980). Familiarity with natal habitat, in particular resource specializations, together with social structure and culturally and vertically transmitted behaviors could possibly contribute towards philopatry for both sexes (Sellas *et al.* 2005; Sargeant & Mann 2009; Möller 2011; Cantor & Whitehead 2013). These processes could lead to assortative mating and maintain divergence at a large-scale between the pelagic and coastal groups, and at a finer scale, within the two groups. Natal habitat preference through diet specializations was suggested as an important mechanism underlying cryptic population structure in terrestrial carnivores (Sacks *et al.* 2005; Pilot *et al.* 2012). Moreover, socio-ecological factors also drove genetic divergence between killer whale populations specialized on distinct prey (Hoelzel *et al.* 1998a; Foote *et al.* 2011). For bottlenose dolphins in the NEA, localized stomach content (Scotland and Bay of Biscay) and stable isotope (Galicia) studies suggested that coastal populations were feeding on estuarine species while demersal or

demerso-pelagic fishes mainly found on the shelf edge (e.g. hake or blue whiting) were the main prey of presumably pelagic bottlenose dolphins (Santos *et al.* 2001b; Spitz *et al.* 2006; Fernandez *et al.* 2011a). The niche specializations of the two groups and the hypotheses described above are investigated in the next chapter.

c) Effective population size estimates: small coastal *vs* large pelagic populations

Effective population sizes were much larger for pelagic than for coastal populations which was consistent with their genetic diversities. As pelagic populations were likely to be very large, N_e estimates for these populations were not reliable (Tallmon *et al.* 2010). In addition, our sample size for the Pelagic Mediterranean population was relatively low for these approaches. For coastal populations, we had a sufficient number of samples (N= 77 and 119) and high precision (25 microsatellites) to get reliable N_e estimates for small populations (N < 500) (Tallmon *et al.* 2010). However, our sampling scheme was not ideal. Two assumptions of both the linkage disequilibrium and Approximate Bayesian Computation methods were likely to be violated: closed populations and discrete generations. For the "no immigration" assumption, the bias could be considered negligible as migration rates were very low and at least for LDNe, migration rates below 5-10% should have little effects on N_e estimates (Waples & England 2011). The "discrete generations" assumption was clearly violated. First, bottlenose dolphins live up to 57 years and are sexually mature between 5 and 14 years (Wells & Scott 1999). Second, our data, collected across a 22-year time period, included multiple cohorts and generations. N_e estimates obtained using samples with overlapping generations are likely to be biased downward (Waples 2010). Nevertheless, a study comparing different N_e estimate methods for a brown bear population showed that the N_e estimate obtained in ONeSAMP on multiple cohorts was similar to the harmonic mean of N_e estimates obtained from single cohorts using another method, the Estimator by Parentage Assignment (Skrbinsek *et al.* 2012). Robinson and Moyer (2013) found that N_e estimates are closer to the per generation N_e when only mature adults are sampled, which resulted to a downward bias of less than 15%. If it is not possible to sample only mature adults, Robinson and Moyer (2013) suggested that as many age classes as possible should be included in the analyses. As our dataset contained multiple age classes and generations, results were likely to

be biased downward. The downward bias depends also on the species' life history. We corrected our estimates for a 15% downward bias (N_ec) as a 10-15% downward bias was observed in a study using LDNe where mature adult bottlenose dolphins of different ages were sampled in Florida (Robin Waples, personal communication). Last but not least, N_e estimated using LDNe related to the effective number of breeders N_b (Waples 2005). Further empirical research is needed on the relationships between N_b and N_e, which could be particularly complex when generations overlap (Waples 2010). Nevertheless, the order of magnitude of the bias should be similar across our dataset. Our N_ec estimates are on par with abundance estimates obtained from surveys in areas inhabited by each of the four populations. The NEA pelagic population abundance estimate from Scotland to Spain was tens of thousands of individuals (Hammond *et al.* 2009; Hammond *et al.* 2013). In the Mediterranean Sea, abundance was estimated to several thousands of individuals (Forcada *et al.* 2004; Gnone *et al.* 2011). According to mark-recapture studies, resident coastal population sizes were likely to be around 600-800 individuals for each of the two populations (Chapter 3 for the Normand-Breton gulf, López 2003; Pesante *et al.* 2008; see review for Ireland in Mirimin *et al.* 2011; Cheney *et al.* 2012). For these two coastal populations, the ratio between effective population sizes and census sizes may be around 5 to 10% based on our N_ec estimates and abundances from mark-recapture studies, which is in the lower end of the range of values found in other species (Palstra & Ruzzante 2008).

d) Management implications

Coastal populations were isolated and their effective population size was small in comparison with pelagic populations. Estimated N_ec (range: ~30 - 80) was close to the value of N_e = 50 under which Mace and Lande (1991) proposed that a population is in a critical state. Low effective population sizes might lead to a low adaptive potential to environmental changes (Hare *et al.* 2011). Ecological adaptation to specific habitats is likely to drive coastal populations' structure (this study, Natoli *et al.* 2005; Rosel *et al.* 2009), which raises concerns about potential impacts from the currently increasing at-sea human activities. Habitat degradation in terms of organic contaminants and noise pollution from boat traffic and constructions (e.g. Pirotta *et al.* 2013) could strongly affect locally adapted coastal populations. In addition, in East England, a genetically differentiated population became

extinct and the estuary was never repopulated (Nichols *et al.* 2007). Several Special Areas of Conservation have been created throughout Europe for the management of bottlenose dolphins, however some important areas for the species still lack conservation measures. Given the vulnerability of small and isolated populations that live within increasingly disturbed environments, we recommend extending the habitat protection of the species in Europe. Moreover, ecotypes should be distinguished in management plans of the species.

e) Ecotype delineation and future directions

Our results showing weaker separation between the pelagic and coastal haplotypes in the NEA found using 324 bp in comparison with 682 bp sequences highlighted the importance of using long fragments of the mitochondrial control region to investigate ecotype delineation in bottlenose dolphins. We therefore recommend the use of long mitochondrial fragments to investigate recent and/or fine-scale genetic structure in delphinids displaying sequence variability levels similar to bottlenose dolphins.

We employed an original approach to define ecotypes, considering Lowry's (2012) definition as groups of ecologically distinct populations. In most studies, ecotypes were first described through diet, morphology or spatial distribution and then linked to genetic differentiation (e.g. Hoelzel *et al.* 1998b; Segura *et al.* 2006; Musiani *et al.* 2007). The latter approach sometimes led to the definition of ecotypes that were subsequently found not to be demographically and genetically isolated units (e.g. caribous Serrouya *et al.* 2012). For cryptic and mobile species for which we have only hints on ecology, genetic data could be an interesting first step in ecotype delineation. Previous distribution and diet studies gave us first clues on the ecological differentiation of coastal and pelagic bottlenose dolphins. Diet specializations and morphological traits of the two ecotypes in the NEA are further investigated in the next chapter.

CHAPTER

6

ECOLOGICAL OPPORTUNITIES AND SPECIALIZATIONS SHAPED GENETIC DIVERGENCE IN A HIGHLY MOBILE MARINE TOP PREDATOR

1) Introduction

Environmental variation is a major driver of evolutionary divergence. It can lead to natural selection on environment-associated traits which can trigger assortative mating, reproductive isolation and ultimately speciation (Schluter 2001; Funk *et al.* 2006). Adaptive divergence can evolve in allopatry when groups of individuals occur in contrasted separated environments (Mayr 1942) or in sympatry and parapatry when they have different ecological niches (Dieckmann & Doebeli 1999; Schluter 2001). In the absence of geographic barriers to gene flow, prey or habitat preferences among groups of individuals can lead to genetic and morphological differentiation. For instance, highly mobile top predators inhabiting neighbouring areas such as boreal forest and taiga/tundra grey wolves specialized on different prey (i.e. resident or migratory) and Galapagos sea lions from two distinct rookeries foraging in benthic and pelagic habitats are genetically differentiated. Their phenotype is also different and related to foraging strategies (Musiani *et al.* 2007; Wolf *et al.* 2008). Similarly, in some birds and post-glacial temperate lake fish species, individuals in sympatry, showing contrasting morphs adapted to different feeding ecology, are at different stages of genetic isolation (Huber *et al.* 2007; Knudsen *et al.* 2010).

In addition, current genetic structure and morphological characters might result from both historical and current ecological conditions. Morphological characters can indeed evolve from very short to evolutionary time scales (e.g. Berner *et al.* 2010; Authier *et al.* 2011). Quaternary glaciation oscillations had a major role in shaping genetic diversity patterns, habitat release during postglacial periods has created ecological opportunities for evolutionary diversification in many species in the Northern Hemisphere (Hewitt 2000). The magnitude of influence of historical *versus* current processes on population structure can vary among species (e.g. Johansson *et al.* 2006; Shikano *et al.* 2010) and both can have an important role. For instance, arctic canids display contrasting patterns of genetic differentiation: non-existent for arctic foxes *versus* strong for grey wolves (Carmichael *et al.* 2007). These patterns are linked to historical processes (i.e. during the last glaciation periods, foxes had a wide distribution while wolves persisted in small refugia) but also to distinct life-histories, social and dispersal behaviors. For instance, while foxes disperse over long-distances following their prey, wolves' ecotypes (resident or migratory) disperse differently depending on their prey.

Preferential dispersal towards a habitat similar to the one of the juvenile life (Davis & Stamps 2004) is likely a mechanism creating and maintaining divergence in highly mobile species. Although this process may be "imprinted" in turtles or fishes (Lohmann *et al.* 2008), social learning of foraging techniques for particular prey or habitat may play a major role in social species having long-term bonds between mothers and calves (Carmichael *et al.* 2007; Musiani *et al.* 2007). Individuals may therefore have higher foraging success in familiar habitat where they can use learned hunting techniques, which might enhance their fitness. This process likely limits gene flow and facilitates local adaptation of ecologically distinct groups of individuals (Kawecki & Ebert 2004).

Cetaceans, which are highly mobile, can show high levels of population structure. This structure is often suggested to be the result of historical processes, social structure or ecological specializations (e.g. Sellas *et al.* 2005; Hoelzel *et al.* 2007). However, genetic studies are rarely correlated with ecology and morphological studies apart for killer whales (reviewed in de Bruyn *et al.* 2013). To understand the forces shaping the structure of diversity, it is essential to integrate ecology and evolutionary approaches (Pelletier *et al.* 2009) in particular for protected cetaceans for which experiments are impossible.

Bottlenose dolphins in the North-East Atlantic form two genetically distinct ecotypes: coastal (i.e. generally occurring in waters less than 40 meters deep) and pelagic (i.e. mainly sighted in deep waters, Chapter 5). They are hierarchically structured with two populations within each ecotype. In the coastal ecotype, the Coastal North population includes individuals sampled around the United-Kingdom and Ireland, and the Coastal South population individuals of the French and Spanish coasts. The pelagic ecotype is divided in the Pelagic Atlantic and Pelagic Mediterranean populations (see Chapter 5 for details). However, the forces having shaped this population structure and the divergence of the two ecotypes are not yet understood. The main objective of this chapter is to address this question using a combination of population genetic and ecological approaches. First, we investigated the most probable population history using Approximate Bayesian Computation and correlated the inferences to past environmental conditions. We tested whether the timeframes of ecotype and population formations are compatible with the creation of new ecological niches. Then, we characterized the morphology and ecology (through the analyses of stable isotope ratios and stomach contents) of the two ecotypes in order to understand how ecotypic differentiation is maintained. By using complementary approaches, we shed light on how environmental

fluctuations and ecological specializations might have shaped genetic and morphological divergences of a marine top predator.

2) Material and methods

a) Genetic inference of the population demographic history

Genetic dataset

Population history analyses were based on 355 biopsy-sampled or stranded bottlenose dolphins analyzed for 25 microsatellites and a 681 base-pair portion of the mitochondrial DNA control region (mtDNA-CR, N = 343) in the previous chapter. Each individual was genetically assigned to one of four populations using spatially-explicit Bayesian clustering analyses (Chapter 5, Figure 6.1).

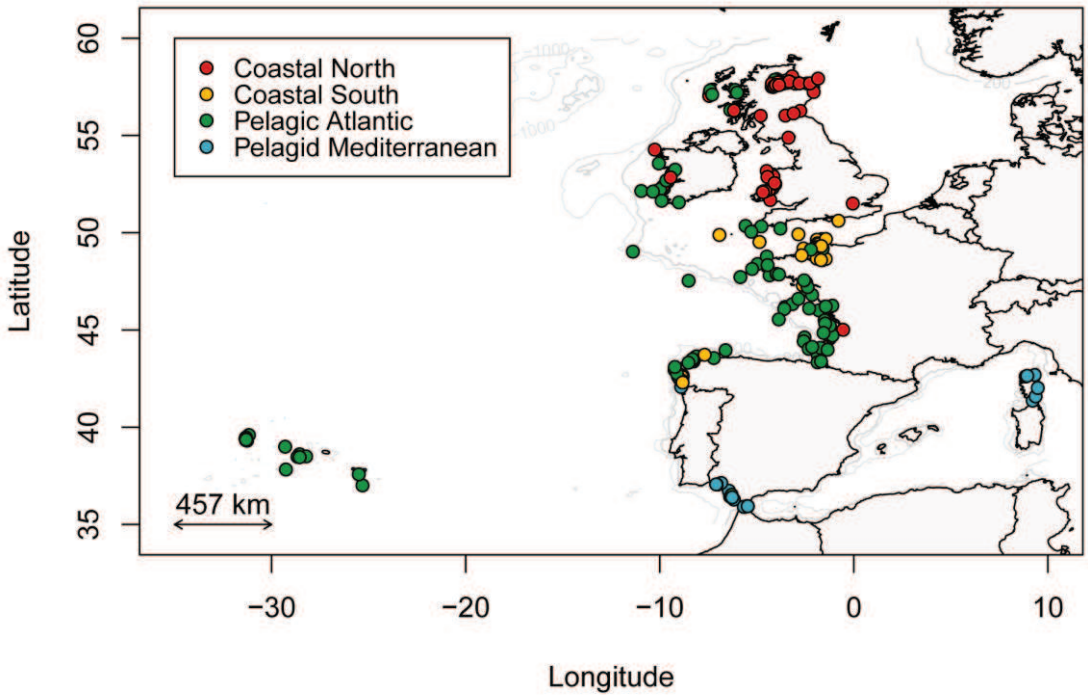


Figure 6.1. Sample locations and genetic populations of bottlenose dolphins included in demographic history analyses.

ABC analysis

We investigated the demographic history best describing the genetic dataset of the combined microsatellite and mtDNA markers using a coalescent-based Approximate Bayesian Computation (ABC) approach (Beaumont *et al.* 2002; Bertorelle *et al.* 2010; Csilléry *et al.* 2010, the general principle of this analysis is presented in Chapter 2.2c). We stratified the procedure in three steps (Figure 6.2): (1) Identify the most likely population tree topologies for our dataset among eleven alternative scenarios describing different potential population topologies (Figure 6.2a); (2) refine the topology of the best tree (Figure 6.2b); (3) and test the occurrence of bottlenecks along the population tree, when each population split from its ancestor (Figure 6.2c).

For each step, an ABC analysis was conducted using the program DIYABC v2.0.4 (Cornuet *et al.* 2014) and include several steps described in Appendix A6.1: (1) Coalescent simulations of 10^6 pseudo-observed datasets (PODs) under each competing scenario and the calculation of summary statistics (SS) describing microsatellites and mtDNA sequences for each POD; (2) Select the best model by estimating the posterior probability of each scenario using a logistic regression on 1% PODs producing SS values closest to the observed ones; (3) Evaluate the confidence in scenario choice by estimating the Type-I and Type-II error rates based on simulated datasets; (4) Estimate the marginal posterior distribution of each parameter based on the best model(s); and finally, (5) Evaluate the goodness-of-fit of the model–posterior parameter distributions combination with the data.

The parameters defining each scenario (i.e. population size, timings of population size changes and splits, and mutation rates) are considered as random variables drawn from prior distributions (Figure 6.2, Appendix A6.2 and A6.3). For each simulation, DIYABC draws a value for each parameter from its prior distribution and performs coalescent simulations to generate a simulated POD with the same number of gene copies and loci per population as observed. It then calculates, for each POD, a set of summary statistics, which are also calculated for the observed data. A Euclidean distance δ is calculated between the statistics obtained for each normalized simulated dataset and those for the observed dataset (Beaumont *et al.* 2002). Details on the mutation model for microsatellite loci and mtDNA locus and the summary statistics used by DIYABC to describe within- and among population genetic diversity are provided in supplementary materials.

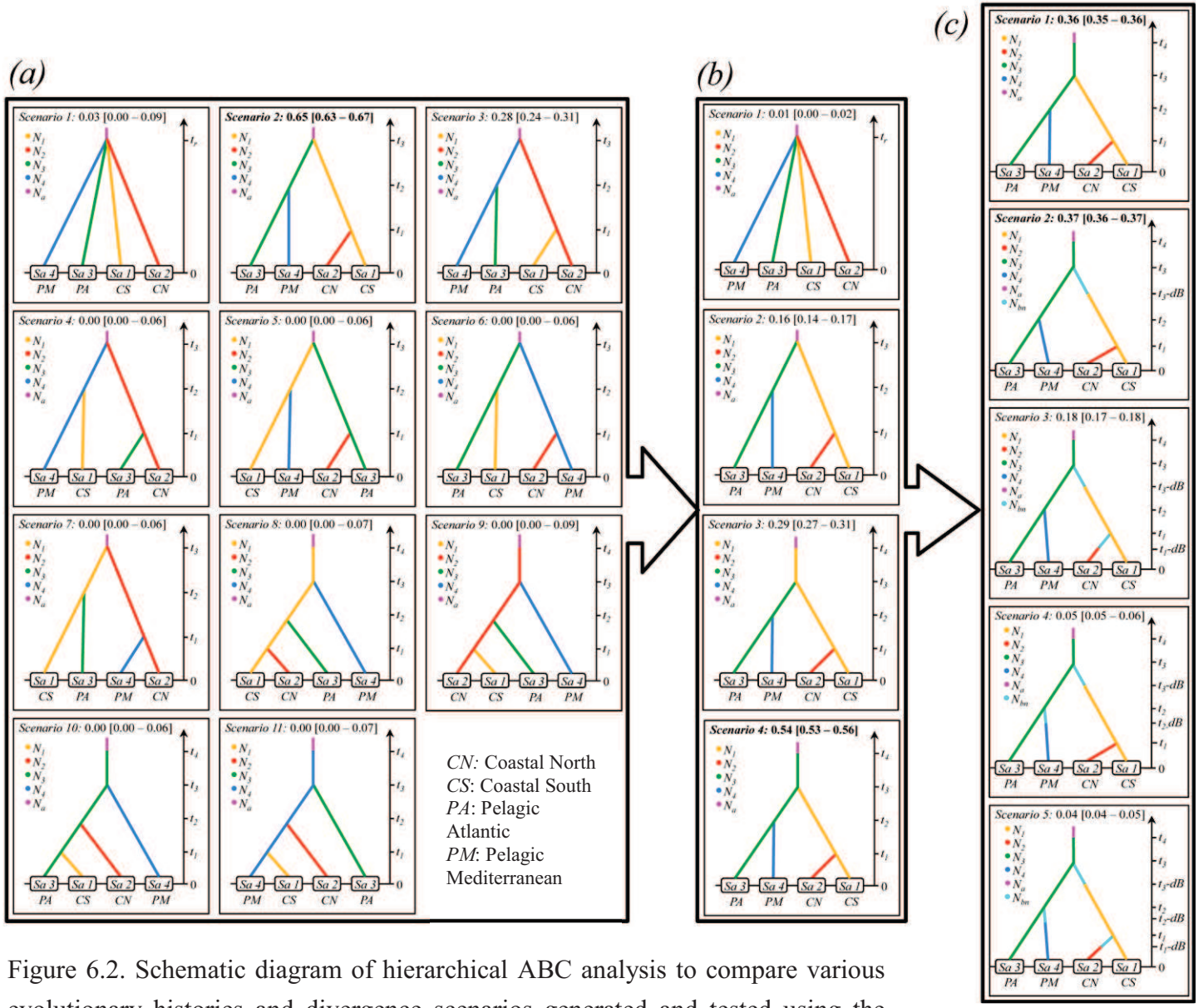


Figure 6.2. Schematic diagram of hierarchical ABC analysis to compare various evolutionary histories and divergence scenarios generated and tested using the program DIYABC.

Model selection procedure and confidence in scenario choice

The posterior probability of each competing scenario was estimated using a polychotomous logistic regression (Cornuet *et al.* 2008; Cornuet *et al.* 2010) on the 1% of simulated datasets closest to the observed dataset (lowest Euclidean distance δ, see above), subject to a linear discriminant analysis as a pre-processing step (to reduce the dimensionality of the data, Estoup *et al.* 2012). The selected scenario was that with the highest posterior probability value with a non-overlapping 95% confidence interval (95%CI). We evaluated the ability of the ABC analysis to discriminate between tested scenarios by analysing simulated datasets with the same number of loci and individuals as our real dataset. Following Cornuet *et al.* (2010), we estimated the Type-I error probability as the proportion of instances in which the selected scenario did not give the highest posterior probability among the competing scenarios, for 500 simulated datasets generated under the best-supported model. We also estimated the Type-II error, by simulating 500 datasets for each alternative scenario and calculating the mean proportion of instances in which the best-supported model was incorrectly selected as the most likely model.

Parameter estimation and model checking

We estimated the posterior distributions of each demographic parameter under the best demographic model, by carrying out local linear regressions on the 1% closest of 10^6 simulated datasets, after the application of a logit transformation to parameter values (Beaumont *et al.* 2002; Cornuet *et al.* 2008). Following Gelman (2003), we evaluated whether the best model-posterior distributions combination was better able to reproduce the observed data compared to the alternative scenarios using the model checking procedure in DIYABC. Model checking was carried out by simulating 1,000 pseudo-observed datasets under each studied model-posterior distribution combination, with sets of parameter values drawn with replacement from the 1,000 sets of the posterior sample. This generated a posterior cumulative distribution function for each simulated summary statistics, from which we were able to estimate the *P*-value of the deviation of the observed value of each statistic from its simulated distribution under the best demographic model.

b) Ecological and morphological characterization of ecotypes

Only ecotypes and not all populations were characterized in terms of ecology and morphometrics because of tissue and data availability. Stranded animals in the English Channel and the Bay of Biscay between 1991 and 2012 (N = 63) were used and included 21 coastal (from the coastal South population apart from 3 individuals that were genetically assigned to the coastal North population) and 42 pelagic individuals (only from the pelagic Atlantic population), and 32 females, 30 males and one individual of unknown gender for which molecular sexing failed (see sampling locations in Appendix A6.4). Morphometric, stable isotope and stomach content analyses were performed on different datasets depending on morphometric measurement, non-decomposed skin and stomach availability. All individuals selected had a length superior to 200 cm to exclude suckling individuals as their nitrogen stable isotope signature is up to one trophic level higher than their mothers as in Fernandez *et al.* (2011a). All statistics were performed in R 3.0.0 (R Core Team 2013).

Morphometric analyses

Ten external morphometric measurements that include the lengths of the appendices and lengths from the rostrum to various body parts (L1 to L10, illustrated in Appendix A6.5) were taken by trained observers of the French stranding network. Morphometric analyses were only performed on individuals for which there were no missing measures and that were not in decomposition to avoid biases ($N_{coastal}$ = 12 and $N_{pelagic}$ = 27 and $N_{females}$ = 20 and N_{males} = 18, $N_{undetermined}$ = 1). As body length was not significantly different between the two ecotypes (Student *t*-test $P = 0.28$), all measurements were standardized over the total body length (L1) to control for different sizes and ages. As there were no trends in ratios from juveniles to adults, all individuals were included in the analyses. First, each ratio was compared between ecotypes using a Student *t*-test or a Mann–Whitney-Wilcoxon test (depending whether the data satisfied normality and homogeneity of variance conditions). Then, a Principal Component Analysis (PCA) was performed using the ade4 package (Dray & Dufour 2007) to test for morphometric segregation between ecotypes. In addition, to test for a division in the dataset, we performed a maximum-likelihood clustering analysis based on Gaussian mixture models with no *a priori* using the mclust package (Fraley *et al.* 2012). We

used the default settings and the best model was selected by BIC (Bayesian Information Criterion). A discriminant function analysis (DFA) was carried out to find the best combination of standardized variables that separate the two ecotypes using the ade4 and MASS packages (Venables & Ripley 2002; Dray & Dufour 2007). Then, we reassigned individuals to each ecotype using the DF and estimated the rate of correct assignment. All analyses were also performed considering males and females separately.

Stable isotope analyses

Stable isotopes of carbon, sulfur and nitrogen were analyzed for 40 skin samples ($N_{coastal}$ = 14 and $N_{pelagic}$ = 26, $N_{females}$ = 24, N_{males} = 15, $N_{undetermined}$ = 1). Sample preparation and analysis are detailed in Chapter 4.2.e and the principle of stable isotope analyses is described in Chapter 2.1.b. Stable isotope values are presented in the conventional δ notation relative to IAEA-1 and IAEA-2, Vienna Pee Dee Belemnite and atmospheric N2 for $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$ values respectively.

Mean differences between coastal and pelagic dolphins and between males and females' $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$ were compared using a Student *t*-test or a Mann–Whitney-Wilcoxon test. Stable isotope niches of the two ecotypes were estimated using multivariate, ellipse-based metrics: SIBER (Stable Isotope Bayesian Ellipses in R, Jackson *et al.* 2011) implemented in the SIAR package (Parnell & Jackson 2011). The standard ellipse area (SEA) is defined by a subsample (40%) of the bivariate data (i.e. the ratios of $\delta^{34}S$ and $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ and $\delta^{13}C$ and $\delta^{15}N$). SEA were corrected for sample size (SEA_c), which is a robust approach when comparing small and unbalanced sample sizes. SEA_B (Bayesian SEA) were calculated using 10^6 posterior draws to statistically compare niche width between ecotypes (Jackson *et al.* 2011). The degree of SEA_c overlap between ecotypes was also estimated. Convex-Hull Areas (polygons encompassing all the data points) were also computed and displayed. As described for morphometric analyses, the mixture model-based clustering analysis in the mclust package was used to estimate the most likely number of clusters and assign individuals to each cluster. Individual assignment probabilities were compared to genetic ecotypes.

Stomach content analysis

Stomach content analysis ($N_{coastal} = 6$, $N_{pelagic} = 24$ for non-empty stomachs) was aimed at describing the diet in terms of prey occurrence, relative abundance and their percentage by ingested biomass, and followed a standard procedure for marine top predators (e.g. Pierce & Boyle 1991). Detailed for bottlenose dolphins in Spitz *et al.* (2006), analytical methods are based on the identification and quantification of prey remains including fish otoliths and bones, cephalopod beaks and crustacean carapaces. Food items were identified to the lowest taxonomic level by using published guides (Clarke 1986; Härkönen 1986; Xavier & Cherel 2009) and our reference collection. Allometric relationships allow reconstructing individual prey body length and mass from otoliths, fish bones, cephalopod beaks or crustacean cephalothorax to provide quantitative description of diets.

The dietary importance of each prey was described by its relative abundance (%N) and by ingested biomass (%M). Relative abundance was defined as the number of individuals of that species found throughout the sample. Biomass was calculated as the product of the average body mass and the number of individuals of the same species in each stomach, summed throughout the entire stomach set. These indices were expressed as percentage frequencies. Ninety-five per cent confidence intervals (95% CI) around the percentages by number and mass were generated for each prey taxon by bootstrap simulations of sampling errors (Santos *et al.* 2001a). The bootstrapping routine was written using R 3.0.0. Random samples were drawn with replacement and the procedure was repeated 1000 times. The lower and upper bounds of the 95%CI were the 25th and 975th values previously ranked in increasing order. The dietary overlap in mass (O) was obtained using the Pianka index (Pianka 1974), which varies from 0 (no overlap) to 1 (complete overlap); values greater than 0.5 are considered to reveal a high overlap.

3) Results

a) Genetic inference of the population demographic history

We used a three-steps procedure to identify the demographic scenario best describing the genetic diversity in the four dolphin populations (Figure 6.2). Among the 11 scenarios tested in the first step (Figure 6.2a), the model SC2 showed the highest fit with the observed data, with a posterior probability (Ppr) of 64.7%, (95% CI: [62.6 – 66.7]). This scenario assumes that Coastal South (CS) population and Pelagic Atlantic (PA) diverged first from an ancestral population, followed by the split of pelagic Mediterranean (PM) from the PA populations and Coastal North (CN) population from CS population. The only other scenario receiving significant support, though much lower than SC2, was SC3 with a Ppr = 28%. This scenario assumes a symmetric hypothesis to SC2 in which PM and CN diverged first from each other, followed by the split of PA from PM and CS from CN. All the other scenarios received less than 3% support from the analysis. Therefore the confidence in the SC2 scenario choice was strong. The evaluation of Type-I error rate (Appendix A6.6) showed that 68.6% of the datasets simulated with SC2 were correctly identified as being produced by SC2. False negative error rates could only be observed with SC3 (16.8%) and with SC1 (9.4%). Estimation of the Type-II error (i.e., false positive) was also very low especially when considering all the alternative scenarios but SC3, with individual error rate lower than 5% (Appendix A6.6). The only scenario producing significant error rate was SC3, with 22.4% of PODs wrongly selected as being generated by SC2. Overall, excluding SC3, our analyses displayed a strong power (88%) to discriminate among the scenarios tested. A model checking of the goodness-of-fit of the scenario–posterior parameter distributions with the real dataset further showed that SC2 was the best at reproducing observed summary statistic values (Appendix A6.6).

The step *b* (Figure 6.2) of the ABC analysis further refined the population tree (SC2) identified in step *a.* Indeed a scenario in which PA is considered as the ancestral population from which CS split, explained significantly better the data with a Ppr = 54% (95%CI: [53.0–55.7]) compared to a scenario in which both PA and CS split from a same common ancestral population (SC2, Ppr =15.7%, 95%CI: [14.2–17.3]). This scenario (SC4 Figure 6.2b)

combined with its posterior parameter distributions provides also a better fit with the observed data (see model checking in Appendix A6.7).

The step *c* in the ABC analysis (Figure 6.2) aimed at testing whether the data contained evidence for a population bottleneck occurring when each population split from their ancestral population. Out of the 5 possibilities tested (Figure 6.2c), the scenarios assuming a bottleneck in the CS population (SC2, Ppr=36.7%, 95%CI: [36.0 –37.4]) or no bottleneck (SC1, Ppr=35.7%, 95%CI: [35.0– 36.5]) received the highest supports, followed by the scenario assuming a bottleneck in the two coastal populations (SC3, Ppr=17.7%, 95%CI: [17.0–18.3]). The other scenarios assuming a potential bottleneck in the PM group (SC4) or in all group (SC5) received significantly lower support (Ppr ≤ 5%, Figure 6.2c and Appendix A6.8). However, the ABC analysis showed weak power to discriminate between the 5 scenarios, and especially between the first three (Appendix A6.8). Interestingly, the scenario best able to reproduce the observed data was SC3, assuming a bottleneck in the two coastal populations (Appendix A6.8 and A6.9).

Considering the two most likely scenarios (SC1 and 2 in Figure 6.2c) and assuming a generation time of 20 years (Taylor *et al.* 2007), the splitting time between the CS and PA groups (*t3,* Figure 6.2c) would be ~10,320 years Before Present (yrBP) (95%CI: [4,300 – 47,800]), between PM and PA (*t2*) about ~7,580 yrBP (95%CI:[2,340 – 22,600]), and between CS and CN (*t1*) ~2,560 yrBP (95%CI: [830 – 6,820]). Estimations of the effective population size were the highest in PA (12,200, 95%CI:[6,360 – 14,700], followed by PM (4,810, 95%CI:[1,500 – 9,200]), CS (2,160, 95%CI:[864 – 3,560]) and CN (1,990, 95%CI:[678 – 3,660], Appendix A6.10).

b) Morphometric analyses

The most likely number of clusters using morphometric data was one. Univariate and multivariate analyses, except the DFA, failed to discriminate ecotypes when considering the whole dataset and sexes separately. The only ratio that was significantly different between coastal and pelagic dolphins was the proportion of the fluke to the total body length but the range of values from the two ecotypes overlapped (mean = 0.21, SD = 0.03 and mean = 0.24,

SD = 0.02 for coastal and pelagic dolphins respectively). Only the DFA allowed to partially discriminating both ecotypes with 74% of dolphins correctly reassigned to their ecotype (0.89 and 0.88 for males and females respectively). The variable that had the strongest weight in the analysis was the fluke length ratio. However, when the variables having the least weights were removed from the analysis, correct assignment rates decreased, which highlighted the need of the complete set of variables to be able to partially discriminate ecotypes.

c) Stable isotope analyses

Pelagic dolphins had higher $\delta^{34}S$ (17.9 ± 0.7‰) and lower $\delta^{15}N$ (14.2 ± 0.8‰) values than coastal dolphins ($\delta^{34}S$ = 14.0 ± 1.0‰, $\delta^{15}N$ = 15.7 ± 0.9‰, $P < 0.01$). There were no significant differences in $\delta^{13}C$ values between the two ecotypes ($\delta^{13}C$ = -16.2 ± 1.1‰ and -16.7 ± 0.6‰ for coastal and pelagic dolphins respectively, $P = 0.06$). No differences were detected between males and females. Isotopic niche spaces of the two ecotypes were distinct. There was no SEA_c overlap when considering $\delta^{34}S$ and $\delta^{15}N$, and $\delta^{34}S$ and $\delta^{13}C$ values (Figures 6.3 and Appendix A6.11a). Little overlap (0.07‰²) was found with $\delta^{13}C$ and $\delta^{15}N$ values (Appendix A6.11b). SEA_B calculated using Bayesian inference indicated a narrower niche width for pelagic dolphins ($SEA_{B\ \delta13C\ –\ \delta34S}$ = 1.3‰², $SEA_{B\ \delta13C\ -\ \delta15N}$ = 1.1‰²) than for coastal dolphins ($SEA_{B\ \delta13C\ –\ \delta34S}$ = 4.0‰², $SEA_{B\ \delta13C\ -\ \delta15N}$ = 3.1‰², $P < 0.01$) despite a larger sample size. Niche width was however not significantly different between the two ecotypes when considering $\delta^{34}S$ and $\delta^{15}N$ values ($SEA_{B\ pelagic}$ = 1.8‰², $SEA_{B\ coastal}$ = 3.0‰², $P = 0.07$). The Bayesian credible intervals based on 100 000 posterior draws can be found in Appendix A6.12a to A6.12c.

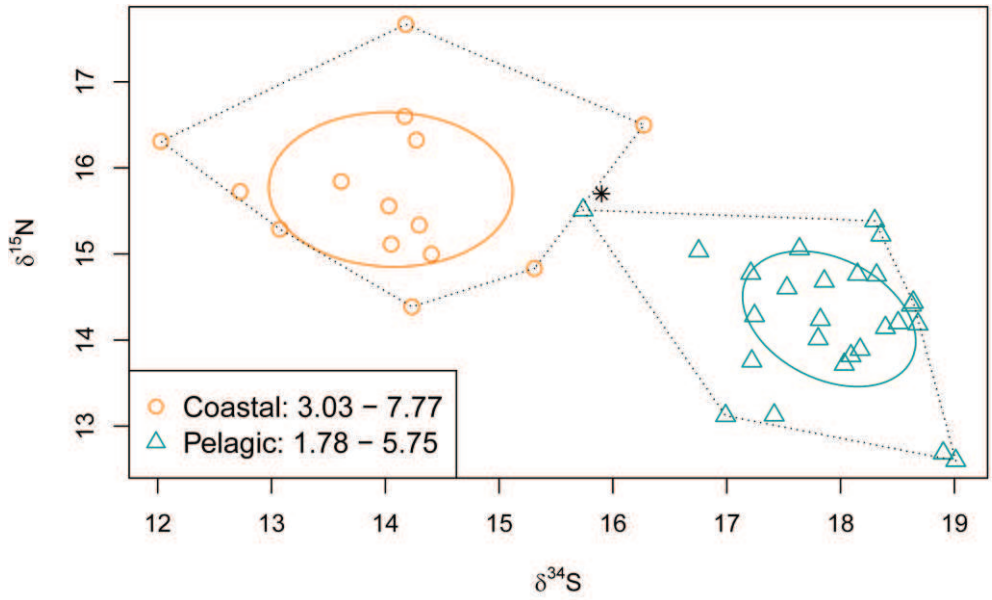


Figure 6.3. $\delta^{34}S$ and $\delta^{15}N$ signatures for genetically determined coastal and pelagic bottlenose dolphins. Solid lines indicate SEA_c and dotted lines Convex Hull Areas and their respective areas values (‰²) are given in the legend. The star indicates the possible migrant.

The most likely number of clusters was two with individuals assigned with high probability to each cluster (Figure 6.4). The isotopic clustering exactly matched the genetic groups apart from one individual which was classified as coastal with stable isotope analyses but was part of the pelagic genetic group. However, this individual was photo-identified with coastal resident dolphins in the English Channel during two years before its death.

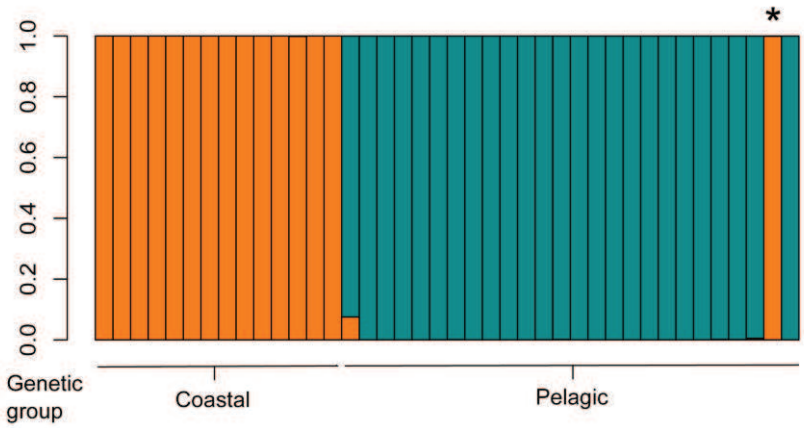


Figure 6.4**.** Barplot of individual assignment probabilities to each of the two isotopic clusters and comparison with genetic groups. Each vertical bar represents one individual. The star indicates the possible migrant.

d) Stomach content analyses

Despite a large prey diversity (30 species including fish, cephalopods and shrimps), one fish species, hake (*Merluccius merluccius*), largely dominated the diet of pelagic dolphins with around 55% of ingested biomass and 25% of the relative abundance (Table 6.1). Mackerel (*Scomber scombrus*) ranked second in term of ingested biomass with 11.4%M. Then, four other species made up a significant proportion of the diet with a relative abundance over 10%N: blue whiting (*Micromesisitius poutassou*), pout (*Trispoterus* spp.), sprat (*Sprattus sprattus*) and scads (*Trachurus* spp.).

The diet of coastal dolphins appeared less diversified (14 species including fish, cephalopods and shrimps) although it was likely linked to a lower sample size. Mullets and pout were the dominant prey with respectively 30% and 31% of ingested biomass. Ammodytidae ranked second in terms of relative abundance (33.7%N) but reached 5% of the ingested biomass.

Thus, the diet of both pelagic and coastal bottlenose dolphins were largely dominated by fish species, however the prey specific composition varied between the two ecotypes. The niche overlap calculated with the Pianka index is particularly low (0.11 by relative abundance and 0.16 by ingested biomass) strengthening the existence of dietary segregation between coastal and pelagic dolphins.

Table 6.1. Diet composition in relative abundance (%N) and ingested biomass (%M) of coastal (N = 6) and pelagic (N=24) bottlenose dolphins. 95% confidence intervals (CI 95%) are given in parentheses.

	Coastal		**Pelagic**	
	%N (CI95%)	%M (CI95%)	%N (CI95%)	%M (CI95%)
Sprattus sprattus			**10.7 (0-28.9)**	0.8 (0-2.9)
Ammodytidae	**33.7 (0-78.5)**	5.2 (0-20.5)	2.9 (0-8.5)	0.1 (0-0.3)
Scomber scombrus			3.6 (0.3-10.2)	**11.6 (0.7-31.6)**
Trachurus spp.	1.1 (0-5.7)	1.1 (0-4.6)	**10.5 (4.0-20.0)**	5.4 (1.8-11.6)
Mugilidae	**35.9 (0-73.4)**	**29.8 (0-63.9)**	1.1 (0.1-2.8)	6.5 (0-19)
Sparidae			3.2 (0-11.2)	1.9 (0-6.5)
Dicentrarchus labrax	3.3 (0-15.4)	6.9 (0-22.6)	4.4 (0.1-13)	5 (0.1-14.9)
Merluccius merluccius			**24.6 (12.9-41)**	**54.6 (28.2-75.5)**
Micromesistius poutassou	5.4 (0-22.7)	0.1 (0-0.4)	**18 (2.4-39.9)**	1.4 (0.4-3)
Trisopterus spp.	5.4 (0-22.6)	**31.1 (5.8-66.9)**	**10.7 (5.9-16.5)**	2.1 (0.9-4.3)
Pollachius spp.	1.1 (0-5.8)	7.6 (0-24.9)		
Other fish	3.3 (0-16.5)	1.3 (0-4.3)	4 (0-10.5)	3.6 (0-9.5)
Loligo spp.	3.3 (0-7.7)	6.1 (0-14.3)	2.1 (0.7-4.2)	6.7 (0.7-17.9)
Other cephalopods	2.2 (0-10.2)	0.1 (0-8.2)	2 (0-5.6)	1 (0.1-10.3)
Shrimp	1.1 (0-4.5)	<0.1 (0-0)	1.9 (0-5.3)	<0.1 (0-0.1)

4) Discussion

a) Ecologically-driven demographic history of bottlenose dolphins in the North-East Atlantic

Ecological conditions had a major role in driving genetic divergence of bottlenose dolphins in the North-East Atlantic (NEA). Approximate Bayesian Computation demographic analyses showed that divergence times between coastal and pelagic and between pelagic Atlantic and Mediterranean bottlenose dolphins correlated with important historical environmental fluctuations. First, we confirmed the often-suggested but never explicitly tested hypothesis of the founding of the coastal populations by the pelagic population (Chapter 5, Natoli *et al.* 2004). The divergence between the two ecotypes occurred between the Last Glacial Maxima and the post-glacial period (10,320 YrBP, 95%IC: 4,300 – 47,800). Therefore, the release of the continental shelf when sea ice retreated after 18,000 YrBP likely led to the colonization of coastal habitats by pelagic dolphins. In addition, although the analysis had relatively low power, this colonization was likely achieved by a small number of

individuals (i.e. a founder effect), which was a common pattern during postglacial periods. More generally, the end of the glaciations in the Northern Hemisphere had a major impact on genetic diversity (Bernatchez & Wilson 1998; Hewitt 2000).

The divergence between pelagic Atlantic and Mediterranean populations occurred later (7,580 YrBP, 95%IC: 2,340 – 22,600), during the Mediterranean "Sapropel period", which was a nutrient-rich period characterized by the deposition of organic-rich sediments on the seafloor. These sediments were formed as a result of increased primary productivity and re-arrangements of water masses linked to increased freshwater inputs generated by high precipitation rates (Calvert *et al.* 1992; Rohling *et al.* 2009). While this phenomena was particularly intense in the Eastern Mediterranean Sea, other major oceanographic and biological changes occurred simultaneously in the Western part around 8,000 YrBP, as a result of increased inflows of Atlantic waters (Rohling *et al.* 1995). These new environmental conditions may have created a productive trophic chain favourable for bottlenose dolphins. Interestingly, these conditions were also likely suitable for harbour porpoises, a small cetacean with high energetic needs. The end of the Sapropel period led to the fragmentation of harbour porpoise populations as waters got too oligotrophic and warm for this cold-waters affiliated species (Fontaine *et al.* 2010; Fontaine *et al. in press*). In contrast, bottlenose dolphins, having a wider range including tropical regions and lower energetic costs (Spitz *et al.* 2012) are still currently observed in the Mediterranean Sea.

These striking links between changes in environmental conditions and genetic divergences indicate that niche opportunities by the release of new habitats or changes in environmental conditions can be a major force creating genetic divergence even in highly mobile top predators.

In contrast, the separation between the two coastal populations was not linked to a particular climatic event (2,560 YrBP, 95%IC: 830 – 6,820). Although it will require further investigations, philopatry or natal-biased dispersal as a result of habitat-specific learned foraging techniques together with social behavior might trigger genetic differentiation as suggested for bottlenose dolphins and other mobile social mammals such as killer whales and wolves (Sellas *et al.* 2005; Hoelzel *et al.* 2007; Musiani *et al.* 2007). Another hypothesis could be the fragmentation of a coastal meta-population as suggested in Nichols *et al.* (2007) who showed that a genetically discrete population in the Humber estuary (South-East

England) disappeared at least 100 years ago. This might be supported by the fact that effective population sizes for coastal populations estimated in DIYABC, which are averaged since their divergence, are 30 to 40 times larger than the ones obtained using LDNe and ONeSAMP which are based on the last few generations (Chapter 5). However as these results could also be linked to methodological differences (i.e. ABC analyses are based on coalescent simulations while LDNe and ONeSAMP use linkage disequilibrium among loci, Tallmon *et al.* 2008; Waples & Do 2008), these comparisons should be considered with caution, and additional evidences are required.

b) Niche specializations maintain genetic divergence between coastal and pelagic ecotypes

As bottlenose dolphins are highly mobile, and the marine environment has no obvious barriers to gene flow, the creation of new coastal niches after the end of the last glacial period is not sufficient to explain the maintenance of genetic divergence between the coastal and pelagic ecotypes. Using two complementary approaches we showed that current ecological niches of pelagic and coastal bottlenose dolphins were highly segregated. Stable isotope signatures and prey species in stomach contents are consistent with a coastal *vs* pelagic habitat/diet segregation (while $\delta^{13}C$ and $\delta^{15}N$ values are lower in offshore areas, $\delta^{34}S$ are higher, Peterson & Fry 1987; Kelly 2000; Chouvelon *et al.* 2012). In addition, prey species exclusively occurring in coastal waters are found in the diet of coastal dolphins while species from the shelf-edge are only found in pelagic individuals. Stable isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ of prey species analyzed in Chouvelon *et al.* (2012) were concordant with values found in the two ecotypes. Prey species have however not been analyzed for sulfur. Pelagic dolphin smaller isotopic niche width is consistent with an offshore more homogeneous environment in contrast to a mosaic of habitats in coastal areas. In addition, although pelagic bottlenose dolphin prey species in stomach contents are diverse, it is dominated by hake, whose large specimens are found mainly along the shelf-edge (Woillez *et al.* 2007). The main prey of both ecotypes are demersal, thus the main differences is the depth where they are found. Hence, we could hypothesize that different hunting techniques might be used and learned to feed in waters of different depth. Bottlenose dolphins might be philopatric or disperse in habitats similar to their natal ones as they will be able to use vertically learned

and/or culturally transmitted hunting techniques (e.g. Cantor & Whitehead 2013) and target familiar prey, which could enhance their foraging success. This hypothesis was suggested for other social mammals (e.g. Carmichael *et al.* 2007), with however rarely direct evidence of diet/foraging segregation such as in our study (but see Pilot *et al.* 2012). In addition, preferential associations with particular individuals that might be influenced by associations during juvenile life (Stanton *et al.* 2011) may also reduce dispersal. Hence, ecological specializations strengthen by social context likely maintain genetic divergence in this highly mobile top predator. However, stability in individual foraging specializations should be further investigated using stable isotope analyses in different dentin layers. We emphasize that stable isotopes could be a powerful tool to understand ecologically-driven cryptic genetic differentiation in a wide-range of taxa as shown in this study and a few others (Wolf *et al.* 2008; Pilot *et al.* 2012).

However, there is some behavioral plasticity in the foraging resources used. Indeed, clustering analyses on stable isotope data matched perfectly the genetic structure except for one individual. This dolphin photo-identified during two years in a coastal area bears coastal-like isotopic signatures but has been genetically identified as belonging to the pelagic group. Current migration rates are very low between ecotypes (Chapter 5). However, as haplotypes are shared between coastal and pelagic dolphins, the individual could possibly be a migrant. Despite niche segregation, some degree of behavioral plasticity might contribute to low levels of gene flow between ecotypes. Ecologically-driven complete genetic isolation could be a long process that might never reach completion or require time (e.g. Berner *et al.* 2010; Foote *et al.* 2013).

c) Absence of strong influence of ecology on external morphological traits

In contrast to our results, pelagic and coastal bottlenose dolphins in other areas of the world showed strong morphological differences. In the North-East Pacific, skulls of coastal bottlenose dolphins had larger rostrum and teeth than pelagic ones, which might be linked to contrasted diets (Perrin *et al.* 2011). In the North-West Atlantic (NWA), coastal individuals were smaller and had proportionally larger flippers possibly to get more manoeuvrability in

shallow estuaries and dissipate heat in warm waters than pelagic individuals inhabiting cold and open waters (Hersh & Duffield 1990). In addition, while coastal dolphins fed mainly on sciaenid fish, pelagic individuals fed on both fish and squid (Mead & Potter 1995). In the NEA, several hypotheses might explain the weak morphological differences. First, haplotype network and coalescent-based estimation of divergence times suggested that differentiation between the two ecotypes occurred more recently in the NEA than in the NWA (Chapter 5, Moura *et al.* 2013), giving less time for morphological divergence. In addition, coastal and pelagic habitats might be less contrasted in the NEA than in the NWA. In the NWA, environmental conditions might be very different between shallow, enclosed and warm estuaries, and cold pelagic waters. In contrast, in the NEA, coastal waters, at the northern range of the species, might be quite similar in terms of temperature and currents than pelagic waters, the main difference being depth. In addition, both ecotypes fed on demersal prey. Thus, lower differential ecological selective pressures might contribute to the lack of morphological differentiation. The only measure which is significantly distinct between the two ecotypes is the fluke width which is larger for pelagic dolphins and may confer more propulsion to dive in deep waters. We could not rule out more subtle differences that could not be captured with our relatively small dataset and differences in skull morphological features which should be investigated in the future (as in Perrin *et al.* 2011).

d) Possible differential stage of speciation in the North Atlantic

We showed that niche creation followed by niche specializations were major drivers of ecotype differentiation in bottlenose dolphins. Further work is needed to investigate ecological specializations among populations within ecotypes. We emphasized that understanding the forces shaping genetic and morphological divergences in highly mobile and cryptic animals is only possible thanks to a combination of evolutionary and ecological approaches. Both provide complementary information on current and past time scales. Similar multi-approach studies could help to shed light on divergence patterns of a lot of other species.

At a large-scale, bottlenose dolphins might show different stages of speciation along its North Atlantic distribution. The speciation process could be on-going and at an early stage

in the NEA and possibly complete in the NWA regarding to complete lineage sorting and strong morphological differentiation (Hersh & Duffield 1990; Hoelzel *et al.* 1998b). Variations in strength of habitat differences, contrasted divergence times or behavioral plasticity may led to different stages of ecologically-driven genetic and morphological divergences for the same species across its range (e.g. for post-glacial fish and killer whales, Berner *et al.* 2010; Knudsen *et al.* 2010; Foote *et al.* 2013). We suggest that environmental opportunity to specialize may be the major factor driving ecological, genetic and morphological divergences.

CHAPTER

7

GENERAL DISCUSSION

1) Synthesis of the results

The objectives of my dissertation were to describe and discuss the forces shaping the social and population structures of bottlenose dolphins in the Normano-Breton gulf (Chapters 3 and 4) and the population structure of the species in the North-East Atlantic (Chapters 5 and 6). Before discussing the fundamental (section 7.2 and 7.3) and applied (section 7.4) implications of our work, I summarize here the main findings of each result chapter.

a) Bottlenose dolphin social, ecological and genetic structures in the Normano-Breton gulf

In Chapter 3, using social structure analyses based on photo-identification data, bottlenose dolphins in the Normano-Breton gulf were shown to have a typical fission-fusion social structure. The majority of individuals showed ephemeral associations but had also a small proportion of long-term relationships. The dolphins of the gulf displayed two different characteristics in comparison to other resident groups. First, group sizes were large (mean = 25) and variable (range: 1 to 100), which may be the result of ecological conditions such as resource predictability and availability. In addition, these dolphins formed three social clusters that were spatially segregated but not completely isolated from each other, i.e. their range largely overlapped and all individuals were indirectly socially connected. Using mark-recapture models, 420 dolphins (95% CI: 331-521) were estimated to occur in the Normano-Breton gulf, making this coastal community one of the largest identified along European coastlines.

In Chapter 4, the social structure results obtained in Chapter 3 were compared with ecological and genetic structures. While a single population was identified using genetics (i.e. a portion of the mitochondrial DNA control region and 25 microsatellites), stable isotopes of nitrogen and sulfur revealed three ecological clusters, consistent with the social clusters defined in Chapter 3. The relative influence of sex, genetic relatedness and ecological

similarity on association patterns was tested. Contrary to my predictions and what is found in most studied bottlenose dolphin communities and many fission-fusion species, individuals did not preferentially associate with kin. Instead they associated with individuals of similar ecology. The absence of influence of relatedness and the large group sizes might be explained by ecological conditions such as the availability and predictability of prey. In addition, as coastal populations may have been more recently founded from a pelagic population in the North-East Atlantic than in other areas of the world (Chapters 5 and 6), bottlenose dolphins of the Normano-Breton gulf may exhibit social organization traits more similar to a pelagic population than a coastal one. Thus, a combination of ecological conditions, in particular resource availability and the absence of predators, individual behavioral preferences and population structure history may have shaped this population social organization.

b) Bottlenose dolphin population structure in the North-East Atlantic

In Chapter 5, the genetic structure of bottlenose dolphins in the North-East Atlantic (NEA) was investigated with an unprecedentedly large sampling size using a portion of the mitochondrial DNA control region and 25 microsatellites. Coastal and pelagic bottlenose dolphins were found to be highly segregated. Their structure was hierarchical, two populations were found within the pelagic (i.e. “Pelagic Atlantic” and “Pelagic Mediterranean”) and the coastal (i.e. “Coastal South” and “Coastal North”) ecotypes. Migration rates between ecotypes and among populations were found to be very low. Philopatry and restricted gene flow were suggested to be the results of ecological specializations and social behavior. Our mitochondrial data were also placed in an Atlantic basin-wide context, which indicated that coastal bottlenose dolphins in the NEA may have been more recently founded by the pelagic population than coastal dolphins of the North-West Atlantic. Estimation of effective population sizes, although having inherent bias, indicated that coastal populations were considerably smaller than pelagic populations. In addition, they were similarly scaled to abundance estimations from photo-identification and large-scale surveys.

In Chapter 6, the aim was to (i) investigate how the population structure and the formation of the coastal and pelagic ecotypes were triggered and (ii) to characterize the two ecotypes using ecological and morphological approaches. Approximate Bayesian Computation demographic analyses confirmed that the coastal populations originated from the pelagic Atlantic population. The times of divergence between the two ecotypes and the pelagic Atlantic and Mediterranean populations were correlated with past environmental changes (i.e. the end of the glaciations and changes in the Mediterranean Sea oceanographic conditions). Thus, ecological opportunity likely triggered genetic divergence. Coastal and pelagic bottlenose dolphin ecological niches (investigated using stomach contents and stable isotopes) are currently highly segregated. Therefore, ecological specializations, which may be strengthened by social behavior, likely maintain genetic divergence. In contrast to other areas in the world, only weak morphological differences were found between the two ecotypes. This may be explained by low contrasts between coastal and pelagic habitats. The main conclusion was that foraging habitats, characterized by different prey communities, are key factors driving ecological, genetic and morphological divergences.

2) Structuring patterns of bottlenose dolphins and other mobile social predators: interaction between ecology, sociality and genetics

a) The central role of ecology in shaping the structure of populations

The results of my PhD showed that ecological structure strongly influenced social and genetic structures. At a fine-scale level, individuals may associate preferentially with individuals having similar ecology, and thus possibly foraging behavior. It is however not possible to unravel if individuals associated because of similar ecology or if they have similar ecology because they associated preferentially according to others traits difficult to measure in cetaceans such as age or previous familiarity, which influenced association patterns of other species (e.g. Wey & Blumstein 2010; Garroway *et al.* 2013). This hypothesis is supported by possible evidences of preferential associations of bottlenose dolphins according to feeding behavior in other parts of the world (e.g. between individuals using similar feeding techniques such as sponges to search for prey on the seafloor or feeding on trawl fishery

discards, Ansmann *et al.* 2012a; Mann *et al.* 2012; Cantor & Whitehead 2013). However, ecological behavior may also be transmitted horizontally between associated individuals (e.g. Whitehead *et al.* 2004; Cantor & Whitehead 2013). For example, depredation behaviors on long-line fisheries of killer whales around Crozet island (Southern Ocean) have most likely been transferred socially between groups that associate the most frequently (Tixier 2012).

On a large-scale, individuals likely mate preferentially with individuals foraging into similar habitats for similar resources. Although reproductive isolation is not complete, coastal and pelagic bottlenose dolphin ecotypes, being segregated at the spatial and trophic levels, are highly genetically differentiated. Ecologically-driven reproductive isolation may also explain the differentiation of other social top predator ecotypes such as killer whales or wolves (Hoelzel *et al.* 2007; Musiani *et al.* 2007) and ecotypes of non-social species such as post-glacial lake fishes (Rundle *et al.* 2000; Knudsen *et al.* 2010; Siwertsson *et al.* 2013).

As the acquisition of resources, both in terms of diet and habitat, has a strong influence on individual reproductive success and fitness (Schoener 1971; Pyke *et al.* 1977; Morse & Fritz 1987; Frey-Roos *et al.* 1995; Pärt 2001; Thayer & Sydeman 2007), it is not surprising that ecology has a major influence on social and genetic structures. Indeed, individual and population niche specializations are considered as important drivers of evolution (Bolnick *et al.* 2003; Knudsen *et al.* 2010).

In this dissertation, I advanced the study of correlation between ecological and genetic structures by investigating how niche specializations and subsequent genetic divergences were triggered. Given the correlation between the divergence times (i.e. between the two ecotypes and the two pelagic populations) and past climatic changes, environmental opportunity likely led to niche specializations. Climatic variations during glaciations shaped genetic diversity of terrestrial species in the Northern Hemisphere (Hewitt 1996, 2000). Likewise, in the marine environment, although evidences are fewer, past changes in oceanographic conditions, in particular sea surface temperatures or resource availability, were suggested to have shaped genetic diversification patterns of other top predators such as common dolphins and minke whales at oceanic scales (Pastene *et al.* 2007; Amaral *et al.* 2012b) or of harbor porpoises at the scale of the NEA (Fontaine *et al. in press*).

On a fine-scale, no evidence is available on how niche specializations among social clusters were created. Nevertheless, several hypotheses could be formulated based on a

literature review. First, similarly to large-scale structuring patterns, habitat or resource heterogeneity is likely an important driver of such segregation. Habitat characteristics were linked to different hunting strategies in other bottlenose dolphin populations around the world (Sargeant *et al.* 2007; Torres & Read 2009; Tyne *et al.* 2012). In the Normano-Breton gulf, habitats are diverse and vary from sandy to rocky sea floors. Although recording feeding techniques visually was not possible in the gulf, dolphins of the different social clusters, differing in their habitat use as confirmed by their stable isotope signatures, may possibly differ in their foraging behavior and prey choice. However, further investigations and data are required to investigate differences in habitat use and diet of individuals belonging to the different clusters. Applying mixing models on stable isotope signatures of bottlenose dolphins of the different clusters and possible prey species may for example help to reveal possible dietary differences (Parnell *et al.* 2010). Another hypothesis could be, as suggested for another marine mammal (i.e. the sea otter), that fine-scale foraging specializations may have arisen as a result of intraspecific competition and low interspecific competition (Estes *et al.* 2003). Bottlenose dolphins are one of the most abundant top predators in the Normano-Breton gulf: there are also small colonies of breeding harbor seals (50 to 70 individuals) and non-breeding grey seals (10 to 20 individuals). Harbor porpoises are encountered only seasonally and sightings of other cetacean species are scarce. In addition, seabirds are also found in the area.

b) Social behavior likely strengthens the influence of ecology on genetic structure

Social behavior, in particular social learning, likely strengthens the link between ecology and genetic structure. It may play an important role in the maintenance of ecological specializations and genetic divergence.

In mammal species such as dolphins, killer whales, wolves, coyotes and sea otters, offspring are found to maintain bonds with their mothers lasting from months for sea otters (Estes *et al.* 2003) to years for bottlenose dolphins (reviewed in Wells & Scott 1999; Connor *et al.* 2000) and life-span for killer whales (Bigg *et al.* 1990). During the calf and juvenile dependency periods, young individuals learn foraging techniques by observation, imitation or assistance (Guinet 1991; Estes *et al.* 2003; Mann & Sargeant 2003; Sargeant & Mann 2009).

These learned foraging techniques might be particularly adapted to specific habitats or prey. Thus, when using these learned techniques on familiar prey or in familiar habitats, individuals will likely have higher foraging success and subsequently possibly higher fitness (reviewed for wolves in Pilot *et al.* 2006). It may therefore be beneficial to stay philopatric or disperse in habitats similar to the natal area. As suggested in Chapters 5 and 6, this process may explain fine-scale genetic structure of highly mobile mammals (Sacks *et al.* 2005; Pilot *et al.* 2006; Musiani *et al.* 2007) including bottlenose dolphins in the North-East-Atlantic (NEA). In addition, in cetaceans, culturally transmitted behavior may also shape large-scale population structure (Whitehead 1998) or within population structure (Kopps *et al.* 2014). For instance, a vertical cultural transmission of a tool-use and habitat-specific feeding technique (i.e. sponging) may explain fine-scale geographical structure of mitochondrial DNA haplotypes of bottlenose dolphins in Western Shark Bay, Australia (Kopps *et al.* 2014).

On a fine-scale, individuals associated non-randomly and similarity in ecology may influence association patterns or *vice versa*. In addition, other traits such as familiarity during juvenile life (Tsai & Mann 2013), or reproductive state (Möller & Harcourt 2008) may contribute to association patterns in this species. These non-random associations with preferential individuals and social group ecological specializations on a fine-scale may reduce dispersal and create genetic structure at a larger-scale (for example between individuals from the Normano-Breton gulf and from Scotland or Wales, United Kindgom). The fact that we did not found any preferential associations between relatives in the Normano-Breton gulf may seem counterintuitive with the latter hypothesis of philopatry. However, we still found pairs of first-order relatives in the Normano-Breton gulf (Chapter 4), randomly spread across the population. As in other populations, offspring, while staying within the population, might tend to decrease associations with their mothers after weaning (Tsai & Mann 2013).

On the other hand, behavioral plasticity in fission-fusion societies may counteract genetic divergences. For instance, the disappearance of the social division between trawler and non-trawler bottlenose dolphins after the limitation of the trawling in Moreton Bay indicated that social structure can be adaptive and resilient to disturbance (Ansmann *et al.* 2012a). Moreover, in another population, dolphins that were likely immigrants were as socially integrated as the local individuals (Wiszniewski *et al.* 2010b). Similarly, the social segregation between local African elephants and translocated individuals decreased over time (Pinter-Wollman *et al.* 2009). Social plasticity may explain the observation of a pelagic

bottlenose dolphin with coastal dolphins in the Normano-Breton gulf (see Chapter 6). While this individual was not included in social structure analyses as it was only identified four times, it associated with resident dolphins. Although migration rates between ecotypes are very low, social plasticity may contribute to the incomplete reproductive isolation between the two ecotypes.

c) Influence of evolutionary history on social structure

As discussed in the previous paragraph, social structure may maintain genetic divergence. In turn, large-scale genetic structure might have an influence on social structure. As hypothesized in Chapter 4, Normano-Breton gulf dolphins may share some social structure characteristics with pelagic dolphins such as the absence of kin structure and large group sizes (Möller 2011) as the two ecotypes shared relatively recent ancestry. The influence of evolutionary history on social structure was so far mostly considered in terms of phylogenetic inertia in the literature. Studies on primate social systems showed that some closely related species have considerable similarity in social structure despite high environmental variations (Di Fiore & Rendall 1994; Ossi & Kamilar 2006; Chapman & Rothman 2009). For instance, despite Cercopithecoids range in a large variety of habitat types and are ecologically diverse, their social structure is highly similar. Shared social characteristics (e.g. female grooming relationships, grouping with kin, coalition, allomothering) appear to be linked to female philopatry (Di Fiore & Rendall 1994). In addition, social structure of *Eulemur* was linked to phylogenetic distance among populations (Ossi & Kamilar 2006). In some birds and equids, phylogenetics also predict variations in social behavior (Prum 1994; Linklater 2000). It is important to mention that we do not aim to minimize or contradict the influence of environment on social structure, which is more than well-established (see the rationale in Chapter 1.2, Alexander 1974; Rubenstein & Wrangham 1986). However, in some species, evolutionary history may also play a role. Evolutionary history has rarely been considered within species and in marine mammal social structure studies. Nonetheless, Beck *et al.* (2012) suggested that phylogenetic signal might influence the social structure of killer whales as their primary social unit, composed by long-term associations, is conserved in the Pacific and Atlantic oceans regardless of the ecology of individuals (i.e. eating fish or mammals). However, the second level of social organization (i.e. associations between these cohesive

groups) is more similar between the Atlantic and Pacific mammal-eating groups and between the Atlantic and Pacific fish-eating groups than between ecologically different groups within oceans, supporting the influence of ecology on social structure. Associations between groups hunting for seals were lower, possibly to limit the chance to be detected by seals (Baird & Dill 1996) while several groups can hunt cooperatively for fish (Nøttestad *et al.* 2002).

Our study also emphasized that it may be important to consider evolutionary history when investigating social structure. As detailed above, the social structure of coastal bottlenose dolphins in the Normano-Breton gulf may be derived from a pelagic population possibly because of a relatively recent divergence between ecotypes (i.e. after the Last Glacial Maxima). Given the rapid responses of fission-fusion societies to environmental conditions in a wide range of species (Wittemyer *et al.* 2005; Smith *et al.* 2008; Henzi *et al.* 2009), we however expect that adaptation to coastal environment would have had largely sufficient time to arise. Thus, some traits might be conserved over large temporal scales. An alternative hypothesis is that similar social structure could be selected in different environments. However, in contrast to pelagic individuals (Wells *et al.* 1999; Silva *et al.* 2008), resource predictability possibly led to the residency of coastal dolphins. Nevertheless, evolutionary history and ecology likely interact in shaping the social structure of coastal bottlenose dolphins.

3) Combination of scales and approaches to study the structure and evolution of populations

As detailed in the introductive chapter, cetaceans are notoriously difficult to study as they spend most of their time underwater and field work is constrained by weather conditions. The results obtained in my dissertation on the social and population structures of bottlenose dolphins in the North-East Atlantic and the underlying driving factors were achieved based on a combination of approaches at different spatial and time scales.

a) Combination of spatial scales

The previous section highlighted the interest of combining studies on the fine and large-scale structures of populations. In short, fine-scale non-random association patterns and ecological structure of social groups such as the ones recorded in the Normano-Breton gulf may explain reduced dispersal and the larger scale genetic structure in the NEA. In addition, genetic structure and evolutionary history in the NEA may help to understand social structure in the Normano-Breton gulf. Thus, even if bottlenose dolphins are extensively studied, combining spatial scales enabled us to explain particular structuring characteristics of bottlenose dolphins in the NEA. In lesser studied species, for which opportunities for comparisons are limited, combining spatial scales may even be more valuable in understanding the forces shaping the structure of populations.

b) Combination of approaches

All the approaches used here have inherent limitations when taken individually and inform on the structure of populations at particular time scales. However, combining these approaches is very powerful to shed light on the ecological and evolutionary mechanisms shaping the structure of populations.

Fine-scale social and population structures of bottlenose dolphins in the Normano-Breton gulf

We used photo-identification, stable isotope and genetic approaches to study the social and population structures of bottlenose dolphins in the Normano-Breton gulf. Photo-identification studies are constrained in time and space. One of the major limitation of stable isotope analyses is the difficulties of interpretation, especially when stable isotope values of baseline trophic levels are unknown as various environmental and biological processes may contribute to isotopic signature variations (reviewed in Ramos & Gonzalez-Solis 2012). Using a combination of the two approaches enabled us to exceed the results that could be achieved should the techniques be applied individually. Indeed, the combination of these data supports site fidelity and the partitioning of habitat and or resource use among social clusters.

Although we found similar social and ecological structures for bottlenose dolphins in the Normano-Breton gulf, a single genetic population was identified, indicating random mating. As ecological niches of the different social clusters are segregated, with overlap still evident, there might not be enough environmental opportunity to facilitate specialization and divergence. We could however not totally exclude too recent or too weak divergences to be detected with our set of markers. Genetic structure is indeed integrated over several generations when working with traditional neutral markers such as mitochondrial DNA or microsatellites. Even in the absence of structure at these markers, adaptive divergence may take place (Thibert-Plante & Hendry 2010).

Although it may not be relevant for the Normano-Breton gulf given the very small spatial scale and the fact that we did not reveal sharp ecological differences, genomics could be a promising approach to detect fine-scale levels of genetic structure. Large numbers of molecular markers, either neutral or under selection, such as Single Nucleotide Polymorphism (SNP, i.e. a variation of a single nucleotide in a DNA sequence) can be obtained from the whole genome using Next-Generation-Sequencing approaches (the applications of SNPs in population genetics is reviewed in Helyar *et al.* 2011). This genome-wide polymorphism can be used to identify loci under selection. Selection can be detected using multiple approaches (Nielsen 2005). For example, loci under selection, can be detected using outlier tests, which detect loci that show greater or lower genetic differentiation among populations than would be expected under neutral conditions (Storz 2005). These approaches are progressively applied in non-model organisms. Several examples in the literature indicated the power of outlier loci to detect structure (i.e. adaptive divergence) among populations that cannot be detected when using neutral loci (i.e. either large numbers of new genomic markers or more conventional markers). For instance, population structure of hake in the North-East Atlantic and the Mediterranean Sea was revisited, and revealed stronger large-scale structure and previously undetected fine-scale population structure when using loci under selection in comparison to neutral loci (Milano *et al.* 2014). Similarly, genetic structure between ecologically distinct stream and shore Okanagan Lake kokanee was only detected with outlier loci (Russello *et al.* 2012). These approaches could be useful to detect adaptive divergence in cetacean species inhabiting heterogeneous environments.

Large-scale population structure of bottlenose dolphins in the North-East Atlantic

The combination of genetic, ecological and morphological approaches was invaluable to understand the evolutionary history and the drivers of ecotype differentiation of bottlenose dolphins in the North-East Atlantic. In addition to identify genetic structure, genetic approaches have also the power to reconstruct the past demographic history of populations thanks to coalescent-based simulations (e.g. Kuhner 2009; Bertorelle *et al.* 2010). In contrast, ecological approaches reveal ecotype structure at recent time scales: a few days for stomach content analyses to several weeks to a few months for stable isotopes in skin (Hicks *et al.* 1985; Browning *et al.* 2014). Morphological characters may evolve from short to evolutionary time scales (e.g. Berner *et al.* 2010; Rode *et al.* 2010). Here, the genetic and the two ecological approaches produce highly consistent results clearly distinguishing bottlenose dolphin coastal and pelagic ecotypes on short to evolutionary time scales. Only a weak morphological segregation was detected between the two ecotypes. Nevertheless, the other approaches allowed for the generation of hypotheses about the lack of a strong morphological differentiation (Chapter 6).

Last but not least, we used clustering methods with no *a priori* to detect fine-scale and large-scale structuring patterns on photo-identification, ecological, morphological and genetic data. Results obtained using objective statistical analyses should be reliable.

Combining approaches – studies beyond bottlenose dolphins in the NEA

Studies using multiple approaches are increasing, bridging the gap between genetics and ecology. Multiple approaches have been successfully employed to define population structure in some other species. For instance, large-scale population structure of bluefin tuna in the Atlantic and the Mediterranean Sea, and the intermingling of adolescents originating from both spawning locations in the North-West Atlantic feeding grounds was determined based on results from a combination of electronic tagging, genetics, stable isotope analyses on otoliths and organochlorine tracers (Block *et al.* 2005; Carlsson *et al.* 2007; Rooker *et al.* 2008a; Rooker *et al.* 2008b; Dickhut *et al.* 2009). The intermingling in North-West Atlantic feeding grounds was higher than previously estimated using only conventional tagging, which was highly dependent on fisheries recaptures (see introduction in Rooker *et al.* 2008b). In

addition, a combination of tracking data and stable isotope analyses helped to understand barriers to gene flow for two genetically differentiated Cook's petrel populations at the extreme North and South of New Zealand (Rayner *et al.* 2011). Gene flow may be limited as a result of habitat specializations during the breeding and non-breeding seasons, breeding asynchrony and philopatry (Rayner *et al.* 2011). The success of multiple approaches can also be illustrated by the integration of ecological behavior (i.e. telemetry) and genetic data that shed light on the possible drivers of cryptic genetic structure of highly mobile carnivores. For instance, for coyotes, fine-scale behavioral studies based on the estimation of relatedness of radio-tracked individuals among packs within and between mountain and valley habitats indicated that dispersal across bioregions was rare (Sacks *et al.* 2005), confirming the results from a larger-scale genetic population structure study (Sacks *et al.* 2004). Similarly, the genetic and morphological differentiation between taiga/tundra and boreal forest wolf ecotypes was related to different migration patterns linked to prey specializations revealed by telemetry (Musiani *et al.* 2007). Evolutionary history analyses can also benefits from a multidisciplinary approach. For instance, the patterns of invasion and adaptation to a cold environment of an invasive ant species were revealed using a combination of genetic analyses, distribution modeling and common-garden experiments (Rey *et al.* 2012).

Stable isotope signatures are progressively used to test the influence of ecology on genetic structure (e.g. for wolves, killer whales and whitefish, Foote *et al.* 2009; Pilot *et al.* 2012; Foote *et al.* 2013; Siwertsson *et al.* 2013). Our study also highlighted their power to link ecological and genetic divergences. Nonetheless, a gradient in baseline stable isotope values or differences between habitats or prey are needed for stable isotopes to be useful. Similar approaches to our study could be employed in any study linking genetic divergence to possible prey specializations. For instance, the studies of coyotes and wolf genetic structure cited above (Sacks *et al.* 2005; Musiani *et al.* 2007) could be complemented by stable isotopes analyses on the samples used for genetics. Stable isotope analyses could be performed on a larger sample size than telemetry (e.g. it was found powerful to infer different habitat use in southern elephant seals, Authier 2011).

Last but not least, our results support the fact that the combination of ecological, morphological and molecular data is essential to delineate species (Raxworthy *et al.* 2007; Crandall 2009) or to investigate on-going speciation. Coastal and pelagic bottlenose ecotypes in the NEA may be at an early stage of on-going speciation (Chapter 6) with ecology being an important driver of reproductive isolation. However, I suggest but cannot explicitly test that reproductive barriers have evolved because of adaptation to different environments. Ecological speciation is indeed difficult to identify, and a lot of studies invoked causation while only demonstrating correlation (Hendry 2009). Thus, caution should be taken when raising ecological speciation especially when experiments are not possible (Hendry 2009).

To conclude this section, I encourage the use of similar multidisciplinary approaches to describe the population structure and unravel eco-evolutionary population histories of any taxa. These integrative studies are also of major interest for conservation as detailed in the next chapter.

4) Implications for conservation

Our work has also practical applications for the management of bottlenose dolphins in the Normano-Breton gulf and the North-East Atlantic, as well as more general recommendations for the delineation of conservation units.

a) Conservation of bottlenose dolphins in the Normano-Breton gulf

While one genetic population was identified in the Normano-Breton gulf, three social and ecological clusters were found. However, all individuals were socially indirectly inter-connected and ecological clusters were not completely segregated. If we consider a management unit as a demographic unit so that population dynamics is driven by local birth and mortality rates (Palsbøll *et al.* 2007), one single management unit may be defined for bottlenose dolphins in the Normano-Breton gulf. However, since external pressures could impact social clusters differently, the persistence of the social and ecological clusters should be carefully monitored in the future. Distinct social or ecological clusters could be integrated in demographic analyses as co-variates (in a similar way to age or sex) to test if any cluster is

on a different demographic trajectory. Indeed, pilot whales social clusters were affected differently by a morbillivirus infection in the South of Spain, i.e. some had lower survival after the disease outbreak while it was not the case for others (Wierucka *et al.* 2014). As social structure and dynamics are strongly influenced by ecological conditions (see rationale in Chapter 1.2), their response to changes in environmental conditions might be rapid (Blumstein 2012). In addition, given the possible correlation between social associations and reproductive success or survival (e.g. Silk 2007; Frère *et al.* 2010a; Silk *et al.* 2010), these changes may impact fitness. Parsons et al. (2009) correlated a decline in social cohesion to a decline in abundance of resident killer whales and suggested that it might be a common response to external stressors. However, negative effects on a population may be undetected when working solely on abundance. For example, culling of wolfs led to low levels of kinship within wolf packs because of the adoption of unrelated individuals. The normal kin structure was restored after the ban of the culling. However, the density did not change significantly, with human-induced mortality being replaced by natural mortality (Rutledge *et al.* 2010). Thus, I recommend a long-term monitoring of both demographic parameters and social organization for the Normano-Breton gulf bottlenose dolphin population.

The Normano-Breton gulf population is genetically distinct from neighboring populations in the United Kingdom and Ireland as well as from individuals of Galicia (see Appendix A7.1). The Normano-Breton gulf is thus an important area for a large and genetically isolated population of bottlenose dolphins. Thus, I recommend the designation of a Special Area of Conservation for this population of bottlenose dolphins, as required under the EU Habitats Directive, where member states are required by law to protect these Annex II species at Favorable Conservation Status (FCS), and whose delineation should be determined by habitat use analyses.

b) Conservation of bottlenose dolphins in the North-East Atlantic

The main finding for conservation of bottlenose dolphins in the NEA is the delineation and characterization of the coastal and pelagic ecotypes, which is new for this area. Although they are not monophyletic for mitochondrial DNA and thus do not represent Evolutionary Significant Units (ESU) *sensu* Moritz (1994, 2002), they are sharply genetically and ecologically distinct, meeting the ecological and genetic definition of ESU of Crandall (2000).

They are likely on two distinct evolutionary trajectories and should be considered as two ESU. I recommend separating coastal and pelagic ecotypes in management plans. Threats are likely different in coastal and pelagic waters, which are affected by different types of human developments (noise pollution and habitat destruction in coastal waters *vs* noise pollution for seismic, oil and gas explorations in pelagic waters). Chemical pollution may be higher in coastal areas. Fishery bycatch may impact both ecotypes although dolphin bycatch issues may be more important in pelagic trawl and drift net fisheries than in smaller coastal fisheries (e.g. Morizur *et al.* 1999; López *et al.* 2003; Rogan & Mackey 2007). Threats may overlap in some areas such as Portugal or Spain where the shelf edge is close to shore. Nevertheless, any impact studies such as bycatch rates or pollutant levels should take the two ecotypes into account. Coastal Special Areas of Conservation are increasingly designated. Our genetic results indicating that the coastal populations are small, weakly diverse and relatively isolated, strengthen the need to protect their habitat. The designation of pelagic protected areas is complex given the likely high mobility of the animals in these areas (Game *et al.* 2009) as well as the monitoring requirements that follow. Nonetheless, habitat modeling work is needed to identify important habitats for genetically distinct pelagic bottlenose dolphins and designated suitable protected areas.

ASCOBANS (Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas), which is a regional agreement on the protection of cetaceans recommended the designation of eighteen Management Units of bottlenose dolphins based on photo-identification, genetics and large-scale boat and aerial surveys (Figure 7.1, ASCOBANS 2013).

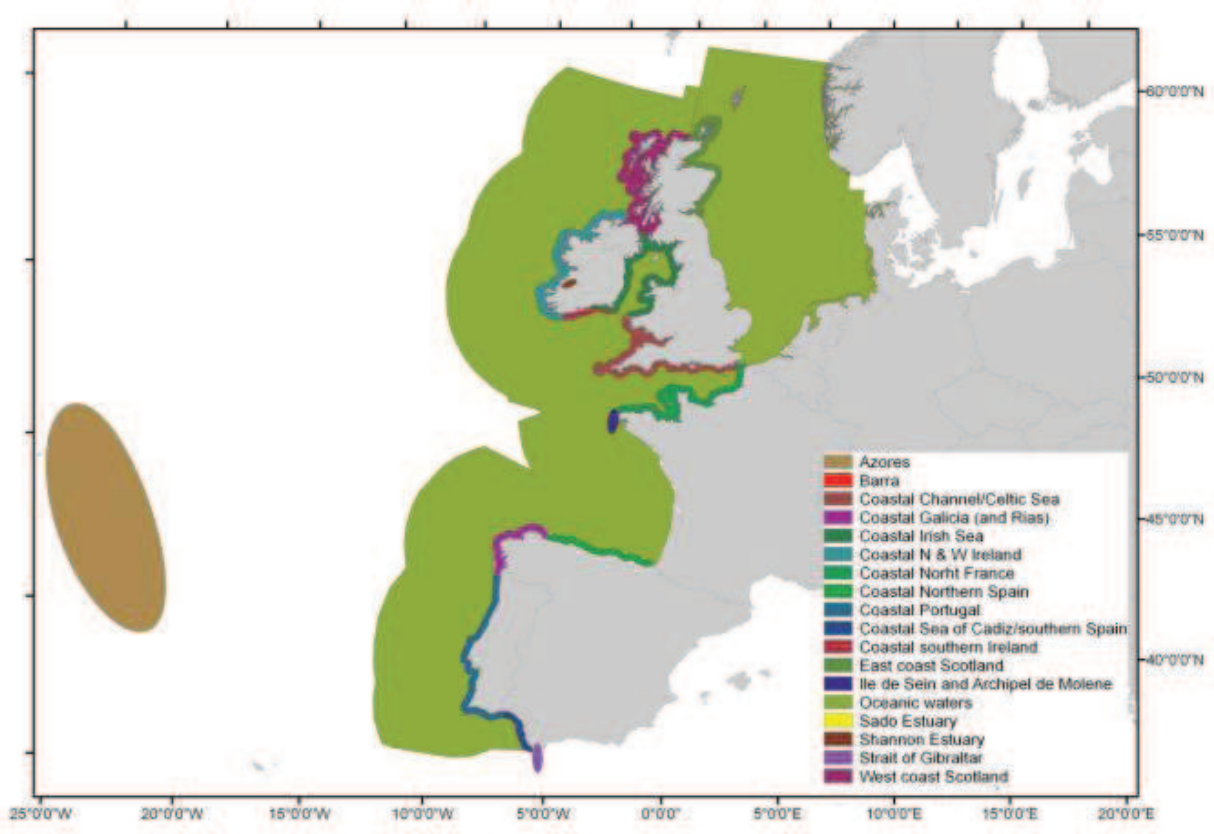


Figure 7.1. Bottlenose dolphin Management Units recommended by the ASCOBANS/OSPAR commission.

This contrasts with the four genetic populations we have identified. Hence, designated management units based on genetic structure only may not be the most effective approach for conservation. I acknowledged in Chapter 5, that finer-scale genetic structure is expected. First, Bayesian clustering analyses may fail to detect low levels of genetic differentiation (Latch *et al.* 2006; Chen *et al.* 2007). Second, although we have a large sample size for this species, we do not have an exhaustive sampling of all resident communities and at least one of them may be genetically isolated (the Shannon population, Ireland, Mirimin *et al.* 2011). For some species such as bottlenose dolphins that show strong site fidelity and fine-scale genetic structure, unless an exhaustive sampling structure is achieved, management units cannot be determined based solely on genetic analyses. Long-term monitoring through photo-identification, complements genetic analyses, and could enable us to reveal demographic independence. Collaborative framework and photo-identification catalogue sharing is also

important to evaluate movements between putative resident and more mobile communities (e.g. in Ireland, and between Ireland and Scotland, O'Brien *et al.* 2009; Robinson *et al.* 2012). I support, as proposed by ASCOBANS, a multidisciplinary approach such as a combination of photo-identification work, ecological tracers and genetic analyses to designate Management Units of bottlenose dolphins.

c) Management implications beyond bottlenose dolphins

I do not want to discredit the relevance of genetic analyses for conservation. Genetic analyses are important to delineate populations, estimate connectivity, effective population sizes and genetic diversity, but it should be recognized that in some cases analyses on neutral markers may fail to detected recent or weak population structure (e.g. Milano *et al.* 2014). Genetic analyses are essential but they should be combined with ecological analyses revealing more recent structuring patterns. Such integrative approaches were successfully employed to designate conservation units for the imperiled salamander and identify the uniqueness of the Walia ibex (Gebremedhin *et al.* 2009; May *et al.* 2011). In these two studies, ecological data were represented by niche modeling. For highly mobile marine animals where it may be difficult to delineate habitats for distinct groups precisely, as detailed in section 7.3, ecological tracers such as stable isotopes, fatty acids or contaminants are efficient alternatives to estimate ecological structure (e.g. Herman *et al.* 2005; Rooker *et al.* 2008a; Dickhut *et al.* 2009; Wilson *et al.* 2012). Whenever possible, a multi-tracers study should be preferred (Ramos & Gonzalez-Solis 2012). For instance, common dolphins in the NEA were identified as forming a single genetic population (Mirimin *et al.* 2009) while ecological tracers revealed significant population structure (Caurant *et al.* 2009). Given the high bycatch pressure for this species, the delineation of appropriate management units is essential for the viability of the species in the NEA (Mannocci *et al.* 2012). On-going genetic structure analyses using outlier loci (SNPs) showed fine-scale genetic structure, relatively coherent with the ecological tracer results (A. Viricel, personal communication). As detailed in section 7.3, population genomics, which allow detecting both neutral genetic structure and adaptive divergence could be a promising tool for conservation in the future, in particular when combined with ecological data (Funk *et al.* 2012).

In addition, analytical approaches that estimate both population genetics and demographic parameters are needed (Palsbøll *et al.* 2007). As detailed in the introduction, one important issue is to determine the level at which populations become demographically independent (Palsbøll *et al.* 2007). For instance, Olsen *et al.* (2014) used an interesting analytical framework to determine harbor seal management units. They used population viability analysis (PVA) to evaluate whether genetic clusters, inferred using Bayesian clustering methods, could be classified as management units based on the "population viability criterion for demographic independence" from Lowe and Allendorf (2010). Inference of management units based on this criterion using genetic data was highly concordant with results of non-genetic methods (habitat use with telemetry), revealing recent and fine-scale structuring patterns that are relevant for management. Although, their approach is appealing, especially as it does not rely on any threshold, it requires detailed life-history data and estimations of census sizes which are not always straightforward to collect in highly mobile or cryptic species. For small populations, individual identification through natural or artificial marks may reveal demographic independence but its applicability will be limited for large and difficult to access populations.

To conclude, I support a multidisciplinary approach to delineate conservation units. There may be no general rule, and the conservation units may be defined case by case using approaches that are the most suitable for the species and area of interest.

5) Perspectives

My dissertation has contributed to fundamental and applied questions on the social and population structures of bottlenose dolphins in the North-East Atlantic. This work offers also several perspectives of studies.

First, it would be relevant for conservation to estimate and compare the contaminant loads (e.g. organic pollutants and trace elements) of coastal and pelagic ecotypes. Bottlenose dolphins are on the top of the trophic chain and bio-accumulate contaminants which can have

immune-toxic and endocrine disruptive effects and may impact their reproductive success (e.g. Reijinders & Aguilar 2002; Schwacke *et al.* 2002; Schwacke *et al.* 2012). Particularly high levels of persistent organic pollutants have been found in bottlenose dolphins in comparison to other cetacean species in the Iberian Peninsula, although sample size was relatively low for this species (Méndez-Fernandez *et al.* 2014). I expect that contaminant levels will vary according to habitat use (e.g. Litz *et al.* 2007; Kucklick *et al.* 2011), with coastal individuals being more exposed to heavy contaminant loads than pelagic ones.

Bottlenose dolphins are often considered as generalist and opportunist feeders according to stomach content analyses. However, it was suggested that individuals may have a degree of specializations (Wells & Scott 1999). This PhD study showed a large-scale level of ecological specializations between ecotypes and fine-scale variation in ecology between social clusters. This may suggest that this species is composed by populations or social groups of specialists. More generally, it was shown that niche width of a generalist population might be the sum of the niche of specialized individuals (Bolnick *et al.* 2003; Bolnick *et al.* 2007). However, with my data, I could not unravel the degree of stability of individual ecology. Dietary specializations at the individual level could be evaluated by analyzing stable isotopes in different dentin layers (e.g. in sperm whales, Mendes *et al.* 2007). It should however be noted that this work could only be performed on dead animals.

The work carried out in this PhD could be extended to the whole North Atlantic (NA) or the whole Atlantic Basin. First, it is suggested that a large undifferentiated bottlenose dolphin population inhabits the NA. However, this hypothesis relies on mitochondrial DNA results only (Quérouil *et al.* 2007, Chapter 5). Pelagic samples of individuals of the North-West Atlantic (NWA) could be genotyped for the same microsatellites used in our study to test for population structure. In addition, NEA coastal populations may have diverged more recently from the pelagic population than NWA coastal populations (see Chapter 6). Population demographic history analyses (using Approximate Bayesian Computation) could be performed on samples from both ecotypes on each side of the Atlantic Basin to estimate and compare the divergence times between ecotypes. In addition, ecological tracers and morphological analyses (on external traits but also on cranial skull features, in particular associated with feeding, Perrin *et al.* 2011) could be carried out. It would then be possible to test my hypotheses about evolutionary history and the differences in morphological differentiation between ecotypes in the NA. In addition, ecological modeling, however limited

by our knowledge on the range of both ecotypes, would be useful to quantify the contrasts between pelagic and coastal habitats from both sides of the Atlantic as I suggested they may be key factors shaping the opportunity to specialize and diverge. Niche modeling is indeed increasingly included in evolutionary studies of terrestrial species to compare the ecological niches of genetically distinct populations (e.g. Gebremedhin *et al.* 2009; May *et al.* 2011) or to contribute to our understanding of evolutionary scenarios (e.g. Rey *et al.* 2012).

This study would also greatly benefit from a genomic approach. I propose to sequence the whole mitogenome and screen thousands of loci on the whole genome such as Single Nucleotide Polymorphisms using Next-Generation-Sequencing (reviewed in Davey *et al.* 2011). The inclusion of these large genomic datasets could improve the power of Approximate Bayesian Computation to identify the most likely demographic scenario. The power of the analyses was relatively limited with our dataset when comparing the most complex scenarios including founder effects (see Chapter 6). Moreover, the results suggest an important influence of environmental factors on genetic divergence. Using a genomic approach, it could be possible to detect loci under selection among the thousands of identified loci between populations. Patterns of parallel evolution and local adaptation have been investigated using these approaches in several species (Hohenlohe *et al.* 2010; Stapley *et al.* 2010; Savolainen *et al.* 2013). For bottlenose dolphins, it would be interesting to test for adaptation and possible parallel evolution in coastal and pelagic waters worldwide using a combination of genomics, ecological and morphological analyses. It could be particularly interesting to include other bottlenose dolphin populations outside the Atlantic, for example include North-East Pacific samples where ecotypes have been identified (Segura *et al.* 2006; Perrin *et al.* 2011) and areas where there might be no ecotype differentiation as suggested for New Zealand (Tezanos-Pinto 2009; Tezanos-Pinto *et al.* 2009). Mapping of loci under selection and identification of possible genes involved may be facilitated by the availability of the whole genome of *Tursiops truncatus* (Lindblad-Toh *et al.* 2011). In addition, bottlenose dolphins in the NEA and NWA may be at different stages of reproductive isolation. This may be the case in other parts of the world, making bottlenose dolphins interesting models to test the influence of ecology on speciation using genome scans together with ecological and phenotypic data (as suggested in Faria *et al.* 2014).

REFERENCES

Alexander RD (1974) The evolution of social behavior. *Annual Review of Ecology and Systematics* **5**, 325-383.

Alter SE, Palumbi SR (2009) Comparing evolutionary patterns and variability in the mitochondrial control region and cytochrome b in three species of baleen whales. *Journal of Molecular Evolution* **68**, 97-111.

Altizer S, Nunn CL, Thrall PH, Gittleman JL, Antonovics J, Cunningham AA, Dobson AP, Ezenwa V, Jones KE, Pedersen AB, Poss M, Pulliam JRC (2003) Social organization and parasite risk in mammals: Integrating theory and empirical studies. *Annual Review of Ecology Evolution and Systematics* **34**, 517-547.

Amaral AR, Beheregaray LB, Bilgmann K, Boutov D, Freitas L (2012a) Seascape genetics of a globally distributed, highly mobile marine mammal: the short-beaked common dolphin (genus *Delphinus*). *Plos One* **7**, e31482.

Amaral AR, Beheregaray LB, Bilgmann K, Freitas L, Robertson KM, Sequeira M, Stockin KA, Coelho MM, Möller LM (2012b) Influences of past climatic changes on historical population structure and demography of a cosmopolitan marine predator, the common dolphin (genus *Delphinus*). *Molecular Ecology* **21**, 4854-4871.

Amos B, Schlotterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. *Science* **260**, 670-672.

Amstrup SC, McDonald TL, Manly BFJ (2005) *Handbook of Capture-Recapture Analysis* Princeton University Press.

Anderson EC, Dunham KK (2008) The influence of family groups on inferences made with the program STRUCTURE. *Molecular Ecology Resources* **8**, 1219-1229.

Ansmann IC, Parra GJ, Chilvers BL, Lanyon JM (2012a) Dolphins restructure social system after reduction of commercial fisheries. *Animal Behaviour* **84**, 575-581.

Ansmann IC, Parra GJ, Lanyon JM, Seddon JM (2012b) Fine-scale genetic population structure in a mobile marine mammal: inshore bottlenose dolphins in Moreton Bay, Australia. *Molecular Ecology* **21**, 4472-4485.

Aplin LM, Farine DR, Morand-Ferron J, Cole EF, Cockburn A, Sheldon BC (2013) Individual personalities predict social behaviour in wild networks of great tits (*Parus major*). *Ecology Letters* **16**, 1365-1372.

Archie EA, Moss CJ, Alberts SC (2006) The ties that bind: genetic relatedness predicts the fission and fusion of social groups in wild African elephants. *Proceedings of the Royal Society B-Biological Sciences* **273**, 513-522.

ASCOBANS (2007) Small cetacean population structure workshop, Bonn, Germany.

ASCOBANS (2013) Draft OSPAR Marine Mammal Indicators. Bycatch Working Group, Warsaw.

Augusto JF, Rachinas-Lopes P, dos Santos ME (2011) Social structure of the declining resident community of common bottlenose dolphins in the Sado Estuary, Portugal. *Journal of the Marine Biological Association of the United Kingdom* **92**, 1773-1782.

Authier M (2011) *Unveiling the at-sea ecology of southern elephant seals from indirect evidence.* PhD dissertation, Université de Poitiers, France.

Authier M, Cam E, Guinet C (2011) Selection for increased body length in Subantarctic fur seals on Amsterdam Island. *Journal of Evolutionary Biology* **24**, 607-616.

Baird RW, Dill LM (1996) Ecological and social determinants of group size in transient killer whales. *Behavioral Ecology* **7**, 408-416.

Baker CS, Florez-Gonzalez L, Abernethy B, Rosenbaum HC, Slade RX, Capella J, Bannister JL (1998) Mitochondrial DNA variation and maternal gene flow among humpback whales of the Southern Hemisphere. *Marine Mammal Science* **14**, 721-737.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37-48.

Barco SG, Swingle WM, McLellan WA, Harris RN, Pabst DA (1999) Local abundance and distribution of bottlenose dolphins (*Tursiops truncatus*) in the nearshore waters of Virginia Beach, Virginia. *Marine Mammal Science* **15**, 394-408.

Barrett-Lennard LG, Smith TG, Ellis GM (1996) A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. *Marine Mammal Science* **12**, 14-27.

Barros NB, Ostrom PH, Stricker CA, Wells RS (2010) Stable isotopes differentiate bottlenose dolphins off West-Central Florida. *Marine Mammal Science* **26**, 324-336.

Bazin E, Glemin S, Galtier N (2006) Population size does not influence mitochondrial genetic diversity in animals. *Science* **312**, 570-572.

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* **73**, 1007-1012.

Bearzi G, Agazzi S, Bonizzoni S, Costa M, Azzellino A (2008) Dolphins in a bottle: abundance, residency patterns and conservation of bottlenose dolphins *Tursiops truncatus* in the semi-closed eutrophic Amvrakikos Gulf, Greece. *Aquatic Conservation-Marine and Freshwater Ecosystems* **18**, 130-146.

Bearzi G, Fortuna C, Reeves R (2012) *Tursiops truncatus* (Mediterranean subpopulation). In: *IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2*.

Beaumont MA, Zhang WY, Balding DJ (2002) Approximate Bayesian Computation in population genetics. *Genetics* **162**, 2025-2035.

Beck S, Kuningas S, Esteban R, Foote AD (2012) The influence of ecology on sociality in the killer whale (*Orcinus orca*). *Behavioral Ecology* **23**, 246-253.

Begon M, Townsend CR, Harper JL (2006) *Ecology from individuals to ecosystems* Blackwell Pub, Malden, MA.

Bejder L, Fletcher D, Brager S (1998) A method for testing association patterns of social animals. *Animal Behaviour* **56**, 719-725.

Bejder L, Samuels A, Whitehead H, Gales N, Mann J, Connor R, Heithaus M, Watson-Capps J, Flaherty C, Krützen M (2006) Decline in relative abundance of bottlenose dolphins exposed to long-term disturbance. *Conservation Biology* **20**, 1791-1798.

Bernatchez L (1997) Mitochondrial DNA analysis confirms the existence of two glacial races of rainbow smelt *Osmerus mordax* and their reproductive isolation in the St Lawrence River estuary (Québec, Canada). *Molecular Ecology* **6**, 73-83.

Bernatchez L, Wilson CC (1998) Comparative phylogeography of nearctic and palearctic fishes. *Molecular Ecology* **7**, 431-452.

Berner D, Roesti M, Hendry AP, Salzburger W (2010) Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Molecular Ecology* **19**, 4963-4978.

Berrow S, O'Brien J, Groth L, Foley A, Voigh K (2012) Abundance estimate of bottlenose dolphins (*Tursiops truncatus*) in the Lower River Shannon candidate Special Area of Conservation, Ireland. *Aquatic Mammals* **38**, 136-144.

Bertorelle G, Benazzo A, Mona S (2010) ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology* **19**, 2609-2625.

Best EC, Seddon JM, Dwyer RG, Goldizen AW (2013) Social preference influences female community structure in a population of wild eastern grey kangaroos. *Animal Behaviour* **86**, 1031-1040.

Best PB, Schell DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. *Marine Biology* **124**, 483-494.

Bigg MA, Olesiuk PK, Ellis GM, Ford JKB, Balcomb KC (1990) Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. *Report of the International Whaling Commission (special issue)* **12**, 383-406.

Bilgmann K, Möller LM, Harcourt RG, Kemper CM, Beheregaray LB (2011) The use of carcasses for the analysis of cetacean population genetic structure: a comparative study in two dolphin species. *Plos One* **6**, e20103.

Birkun A (2012) *Tursiops truncatus ssp. ponticus*. In: *IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2*.

Block BA, Teo SLH, Walli A, Boustany A, Stokesbury MJW, Farwell CJ, Weng KC, Dewar H, Williams TD (2005) Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* **434**, 1121-1127.

Blumstein DT (2012) Social behaviour. In: *Behavioural responses to a changing world* (eds. Wong B, Candolin U), pp. 119-128. Oxford University Press, Oxford.

Bolnick DI (2001) Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. *Nature* **410**, 463-466.

Bolnick DI, Svanback R, Araujo MS, Persson L (2007) Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 10075-10079.

Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist* **161**, 1-28.

Bouveroux T, Mallefet J (2010) Social structure of bottlenose dolphins, *Tursiops truncatus*, in Panama City, Florida. *Journal of the Marine Biological Association of the United Kingdom* **90**, 1685-1692.

Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). *Molecular Ecology* **14**, 2389-2402.

Boyd IL, Arnould JPY, Barton T, Croxall JP (1994) Foraging behavior of Antarctic fur seals during periods of contrasting prey abundance. *Journal of Animal Ecology* **63**, 703-713.

Brinkmann B, Klintschar M, Neuhuber F, Huhne J, Rolf B (1998) Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. *American Journal of Human Genetics* **62**, 1408-1415.

Browning NE, Dold C, I-Fan J, Worthy GAJ (2014) Isotope turnover rates and diet-tissue discrimination in skin of *ex situ* bottlenose dolphins (*Tursiops truncatus*). *Journal of Experimental Biology* **217**, 214-221.

Bruck JN (2013) Decades-long social memory in bottlenose dolphins. *Proceedings of the Royal Society B-Biological Sciences* **280**, 20131726.

Burnham KP, Anderson DR (2002) *Model selection and multimodel inference. A practical information-theoretic approach* Springer, New York.

Butts C (2013) sna: Tools for Social Network Analysis. R package version 2.3-1. http://CRAN.R-project.org/package=sna.

Cairns SJ, Schwager SJ (1987) A comparison of association indexes. *Animal Behaviour* **35**, 1454-1469.

Calvert SE, Nielsen B, Fontugne MR (1992) Evidence from nitrogen isotope ratios for enhanced productivity during formation of eastern Mediterranean sapropels. *Nature* **359**, 223-225.

Cantor M, Wedekin LL, Guimaraes PR, Daura-Jorge FG, Rossi-Santos MR, Simoes-Lopes PC (2012) Disentangling social networks from spatiotemporal dynamics: the temporal structure of a dolphin society. *Animal Behaviour* **84**, 641-651.

Cantor M, Whitehead H (2013) The interplay between social networks and culture: theoretically and among whales and dolphins. *Philosophical Transactions of the Royal Society B-Biological Sciences* **368**, 20120340.

Carlsson J, McDowell JR, Carlsson JEL, Graves JE (2007) Genetic identity of YOY bluefin tuna from the eastern and Western Atlantic spawning areas. *Journal of Heredity* **98**, 23-28.

Carmichael LE, Krizan J, Nagy JA, Fuglei E, Dumond M, Johnson D, Veitch A, Berteaux D, Strobeck C (2007) Historical and ecological determinants of genetic structure in arctic canids. *Molecular Ecology* **16**, 3466-3483.

Carstensen J, Henriksen OD, Teilmann J (2006) Impacts of offshore wind farm construction on harbour porpoises: acoustic monitoring of echolocation activity using porpoise detectors (T-PODs). *Marine Ecology Progress Series* **321**, 295-308.

Carter KD, Seddon JM, Frère CH, Carter JK, Goldizen AW (2013) Fission-fusion dynamics in wild giraffes may be driven by kinship, spatial overlap and individual social preferences. *Animal Behaviour* **85**, 385-394.

Caurant F, Chouvelon T, Lahaye V, Mendez-Fernandez P, Rogan E, Spitz J, Ridoux V (2009) The use of ecological tracers for discriminating dolphin population structure: the case of the short-beaked common dolphin *Delphinus delphis* in European Atlantic waters. *International Whaling Commission* **SC/61/SM34**.

Certain G, Ridoux V, van Canneyt O, Bretagnolle V (2008) Delphinid spatial distribution and abundance estimates over the shelf of the Bay of Biscay. *Ices Journal of Marine Science* **64**, 656-666.

Chapman CA, Rothman JM (2009) Within-species differences in primate social structure: evolution of plasticity and phylogenetic constraints. *Primates* **50**, 12-22.

Chapman CA, Wrangham RW, Chapman LJ (1995) Ecological constraints on group size: an analysis of spider monkey and chimpanzee subgroups. *Behavioral Ecology and Sociobiology* **36**, 59-70.

Charlton-Robb K, Gershwin LA, Thompson R, Austin J, Owen K, McKechnie S (2011) A new dolphin species, the Burrunan dolphin *Tursiops australis* sp. nov., endemic to Southern Australian coastal waters. *Plos One* **6**, e24047.

Chaverri G (2010) Comparative social network analysis in a leaf-roosting bat. *Behavioral Ecology and Sociobiology* **64**, 1619-1630.

Chen C, Durand E, Forbes F, Francois O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes* **7**, 747-756.

Cheney B, Culloch RM, Durban JW, Elwen S, Islas V, Costa M, Eisfeld SM, Evans PGH, Hammond PS, Ingram S, Janik VM, Mandleberg L, Quick N, Reid J, Reid RJ, Robinson KP, Stevick PT, Thompson PM, Wilson B (2012) Integrating multiple data sources to assess the distribution and abundance of bottlenose dolphins *Tursiops truncatus* in Scottish waters. *Mammal Review* **42**, 71-88.

Cherel Y, Ducatez S, Fontaine C, Richard P, Guinet C (2008) Stable isotopes reveal the trophic position and mesopelagic fish diet of female southern elephant seals breeding on the Kerguelen Islands. *Marine Ecology Progress Series* **370**, 239-247.

Chessel D, Dufour AB, Thioulouse J (2004) The ade4 package-I: one-table methods. *R News* **4**, 5-10.

Chico Portillo C, Jiménez Torres C, Pérez S, Verbogh P, Gauffier P, Esteban R, Giménez J, Santos Vega ME, Ezequiel C, de Stephanis R (2011) Survival rate, abundance and residency of bottlenose dolphins (*Tursiops truncatus*) in the Strait of Gibraltar. In: *25th Conference of the European Cetacean Society. Long-term datasets on marine mammals: learning from the past to manage the future. 21st-23rd March 2011*, Cadiz, Spain.

Chilvers BL, Corkeron PJ (2001) Trawling and bottlenose dolphins' social structure. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 1901-1905.

Chilvers BL, Corkeron PJ (2002) Association patterns of bottlenose dolphins (*Tursiops aduncus*) off Point Lookout, Queensland, Australia. *Canadian Journal of Zoology* **80**, 973-979.

Chouvelon T, Spitz J, Caurant F, Mendez-Fernandez P, Chappuis A, Laugier F, Le Goff E, Bustamante P (2012) Revisiting the use of delta ^{15}N in meso-scale studies of marine food webs by considering spatio-temporal variations in stable isotopic signatures - The case of an open ecosystem: The Bay of Biscay (North-East Atlantic). *Progress in Oceanography* **101**, 92-105.

Clarke MR (1986) *A handbook for the identification of cephalopod beaks*, Oxford.

Clobert J, Lebreton JD, Allaine D (1987) A general approach to survival rate estimation by recaptures or resightings of marked birds. *Ardea* **75**, 133-142.

Clutton-Brock T (2007) Sexual selection in males and females. *Science* **318**, 1882-1885.

Clutton-Brock T (2009) Cooperation between non-kin in animal societies. *Nature* **462**, 51-57.

Clutton-Brock TH, Gaynor D, Kansky R, MacColl ADC, McIlrath G, Chadwick P, Brotherton PNM, O'Riain JM, Manser M, Skinner JD (1998) Costs of cooperative behaviour in suricates (*Suricata suricatta*). *Proceedings of the Royal Society B-Biological Sciences* **265**, 185-190.

Clutton-Brock TH, Parker GA (1992) Potential reproductive rates and the operation of sexual selection. *Quarterly Review of Biology* **67**, 437-456.

Coltman DW, Pilkington JG, Pemberton JM (2003) Fine-scale genetic structure in a free-living ungulate population. *Molecular Ecology* **12**, 733-742.

Connolly RM, Guest MA, Melville AJ, Oakes JM (2004) Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia* **138**, 161-167.

Connor RC, Heithaus MR, Barre LM (1999) Superalliance of bottlenose dolphins. *Nature* **397**, 571-572.

Connor RC, Smolker RA, Richards AF (1992) Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp.). *Proceedings of the National Academy of Sciences of the United States of America* **89**, 987-990.

Connor RC, Watson-Capps JJ, Sherwin WB, Krützen M (2011) A new level of complexity in the male alliance networks of Indian Ocean bottlenose dolphins (*Tursiops* sp.). *Biology Letters* **7**, 623-626.

Connor RC, Wells R, Mann J, Read A (2000) The bottlenose dolphin: social relationships in a fission-fusion society. In: *Cetacean Societies: Field studies of whales and dolphins* (eds. Mann J, Connor RC, Tyack P, Whitehead H), pp. 91-126. University of Chicago Press.

Corander J, Siren J, Arjas E (2008) Bayesian spatial modeling of genetic population structure. *Computational Statistics* **23**, 111-129.

Cornuet JM, Pudlo P, Veyssier J, Dehne-Garcia A, Gautier M, Leblois R, Marin JM, Estoup A (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics*.

Cornuet JM, Ravigne V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). *Bmc Bioinformatics* **11**, 401.

Cornuet JM, Santos F, Beaumont MA, Robert CP, Marin JM, Balding DJ, Guillemaud T, Estoup A (2008) Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. *Bioinformatics* **24**, 2713-2719.

Coulon A, Guillot G, Cosson JF, Angibault JMA, Aulagnier S, Cargnelutti B, Galan M, Hewison AJM (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular Ecology* **15**, 1669-1679.

Couzin ID (2006) Behavioral ecology: Social organization in fission-fusion societies. *Current Biology* **16**, R169-R171.

Crandall KA (2009) A multifaceted approach to species conservation. *Animal Conservation* **12**, 105-106.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution* **15**, 290-295.

Crawford AM, Cuthbertson RP (1996) Mutations in sheep microsatellites. *Genome Research* **6**, 876-879.

Croft DP, James R, Ward AJW, Botham MS, Mawdsley D, Krause J (2005) Assortative interactions and social networks in fish. *Oecologia* **143**, 211-219.

Croft DP, Krause J, Darden SK, Ramnarine IW, Faria JJ, James R (2009) Behavioural trait assortment in a social network: patterns and implications. *Behavioral Ecology and Sociobiology* **63**, 1495-1503.

Csilléry K, Blum MGB, Gaggiotti OE, Francois O (2010) Approximate Bayesian Computation (ABC) in practice. *Trends in Ecology & Evolution* **25**, 410-418.

Currey RJC, Dawson SM, Slooten E (2009a) An approach for regional threat assessment under IUCN Red List criteria that is robust to uncertainty: The Fiordland bottlenose dolphins are critically endangered. *Biological Conservation* **142**, 1570-1579.

Currey RJC, Dawson SM, Slooten E, Schneider K, Lusseau D, Boisseau OJ, Haase P, Williams JA (2009b) Survival rates for a declining population of bottlenose dolphins in Doubtful Sound,

New Zealand: an information theoretic approach to assessing the role of human impacts. *Aquatic Conservation-Marine and Freshwater Ecosystems* **19**, 658–670.

Curry BE, Smith J (1998) Phylogeographic structure of the bottlenose dolphin (*Tursiops truncatus*): stock identification and implications for management. In: *Molecular genetics of Marine Mammals.* (eds. Dizon AE, Chivers SJ, Perrin WF), pp. 227-247. The Society for Marine Mammalogy, Lawrence, Kansas.

Dalebout ML, Robertson KM, Frantzis A, Engelhaupt D, Mignucci-Giannoni AA, Rosario-Delestre RJ, Baker CS (2005) Worldwide structure of mtDNA diversity among Cuvier's beaked whales (*Ziphius cavirostris*): implications for threatened populations. *Molecular Ecology* **14**, 3353-3371.

Daura-Jorge FG, Cantor M, Ingram SN, Lusseau D, Simoes-Lopes PC (2012) The structure of a bottlenose dolphin society is coupled to a unique foraging cooperation with artisanal fishermen. *Biology Letters* **8**, 702-705.

Daura-Jorge FG, Ingram SN, Simoes-Lopes PC (2013) Seasonal abundance and adult survival of bottlenose dolphins (*Tursiops truncatus*) in a community that cooperatively forages with fishermen in southern Brazil. *Marine Mammal Science* **29**, 293-311.

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* **12**, 499-510.

Davis JM, Stamps JA (2004) The effect of natal experience on habitat preferences. *Trends in Ecology & Evolution* **19**, 411-416.

de Bruyn PJN, Tosh CA, Terauds A (2013) Killer whale ecotypes: is there a global model? *Biological Reviews* **88**, 62-80.

De Niro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid-synthesis. *Science* **197**, 261-263.

de Stephanis R, Cornulier T, Verborgh P, Sierra JS, Gimeno NP, Guinet C (2008a) Summer spatial distribution of cetaceans in the Strait of Gibraltar in relation to the oceanographic context. *Marine Ecology Progress Series* **353**, 275-288.

de Stephanis R, Garcia-Tiscar S, Verborgh P, Esteban-Pavo R, Perez S, Minvielle-Sebastia L, Guinet C (2008b) Diet of the social groups of long-finned pilot whales (*Globicephala melas*) in the Strait of Gibraltar. *Marine Biology* **154**, 603-612.

de Stephanis R, Verborgh P, Perez S, Esteban R, Minvielle-Sebastia L, Guinet C (2008c) Long-term social structure of long-finned pilot whales (*Globicephala melas*) in the Strait of Gibraltar. *Acta Ethologica* **11**, 81-94.

Defran RH, Weller DW (1999) Occurrence, distribution, site fidelity, and school size of bottlenose dolphins (*Tursiops truncatus*) off San Diego, California. *Marine Mammal Science* **15**, 366-380.

Defran RH, Weller DW, Kelly DL, Espinosa MA (1999) Range characteristics of Pacific coast bottlenose dolphins (*Tursiops truncatus*) in the Southern California Bight. *Marine Mammal Science* **15**, 381-393.

Dekker D, Krackhardt D, Snijders TAB (2007) Sensitivity of MRQAP tests to collinearity and autocorrelation conditions. *Psychometrika* **72**, 563-581.

Di Fiore A, Rendall D (1994) Evolution of social organization - a reappraisal for primates by using phylogenetic methods. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 9941-9945.

Dickhut RM, Deshpande AD, Cincinelli A, Cochran MA, Corsolini S, Brill RW, Secor DH, Graves JE (2009) Atlantic bluefin tuna (*Thunnus thynnus*) population dynamics delineated by organochlorine tracers. *Environmental Science & Technology* **43**, 8522-8527.

Dieckmann U, Doebeli M (1999) On the origin of species by sympatric speciation. *Nature* **400**, 354-357.

Dionne M, Caron F, Dodson JJ, Bernatchez L (2008) Landscape genetics and hierarchical genetic structure in Atlantic salmon: the interaction of gene flow and local adaptation. *Molecular Ecology* **17**, 2382-2396.

Doebeli M, Dieckmann U (2003) Speciation along environmental gradients. *Nature* **421**, 259-264.

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* **22**, 1-20.

Dray S, Dufour AB, Chessel D (2007) The ade4 package-II: two-table and K-table methods. *R News* **7**, 47-52.

Duffield DA, Ridgeway SH, Cornell LH (1983) Hematology distinguishes coastal and offshore forms of dolphins (*Tursiops*). *Canadian Journal of Zoology* **61**, 930- 933.

Dunshea G, Barros NB, McCabe EJB, Gales NJ, Hindell MA, Jarman SN, Wells RS (2013) Stranded dolphin stomach contents represent the free-ranging population's diet. *Biology Letters* **9**, 20121036.

Durand E, Chen E, Francois O (2009a) Tess version 2.3 - Reference Manual.

Durand E, Jay F, Gaggiotti OE, Francois O (2009b) Spatial inference of admixture proportions and secondary contact zones. *Molecular Biology and Evolution* **26**, 1963-1973.

Earl DA, Vonholdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359-361.

Efron B, Stein C (1981) The jackknife estimate of variance. *Annals of Statistics* **9**, 586-596.

Emlen ST, Oring LW (1977) Ecology, sexual selection and the evolution of mating systems. *Science* **197**, 215-223.

Estes JA, Riedman ML, Staedler MM, Tinker MT, Lyon BE (2003) Individual variation in prey selection by sea otters: patterns, causes and implications. *Journal of Animal Ecology* **72**, 144-155.

Estoup A, Angers B (1998) Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: *Advances in molecular ecology* (ed. Carvalho GR), pp. 55-86. IOS press, Amsterdam.

Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology* **19**, 4113-4130.

Estoup A, Lombaert E, Marin JM, Guillemaud T, Pudlo P, Robert CP, Cornuet JM (2012) Estimation of demo-genetic model probabilities with Approximate Bayesian Computation using linear discriminant analysis on summary statistics. *Molecular Ecology Resources* **12**, 846-855.

Estrada JA, Rice AN, Natanson LJ, Skomal GB (2006) Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology* **87**, 829-834.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611-2620.

Excoffier L, Estoup A, Cornuet JM (2005) Bayesian analysis of an admixture model with mutations and arbitrarily linked markers. *Genetics* **169**, 1727-1738.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes - application to human mitochondrial-DNA restriction data. *Genetics* **131**, 479-491.

Eyre-Walker A (2000) Do mitochondria recombine in humans? *Philosophical Transactions of the Royal Society B-Biological Sciences* **355**, 1573-1580.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **164**, 1567-1587.

Faria R, Renaut S, Galindo J, Pinho C, Melo-Ferreira J, Melo M, Jones F, Salzburger W, Schluter D, Butlin R (2014) Advances in Ecological Speciation: an integrative approach. *Molecular Ecology* **23**, 513-521.

Fauchald P, Erikstad KE (2002) Scale-dependent predator-prey interactions: the aggregative response of seabirds to prey under variable prey abundance and patchiness. *Marine Ecology Progress Series* **231**, 279-291.

Fernandez R, Garcia-Tiscar S, Santos MB, Lopez A, Martinez-Cedeira JA, Newton J, Pierce GJ (2011a) Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: evidence of resource partitioning? *Marine Biology* **158**, 1043-1055.

Fernandez R, Santos MB, Pierce GJ, Llavona A, Lopez A, Silva MA, Ferreira M, Carrillo M, Cermeno P, Lens S, Piertney SB (2011b) Fine-scale genetic structure of bottlenose dolphins, *Tursiops truncatus*, in Atlantic coastal waters of the Iberian Peninsula. *Hydrobiologia* **670**, 111-125.

Fontaine MC, Baird SJE, Piry S, Ray N, Tolley KA, Duke S, Birkun A, Ferreira M, Jauniaux T, Llavona A, Ozturk B, Ozturk AA, Ridoux V, Rogan E, Sequeira M, Siebert U, Vikingsson GA, Bouquegneau JM, Michaux JR (2007) Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. *Bmc Biology* **5**, 30.

Fontaine MC, Roland K, Calves I, Austerlitz F, Tolley KA, Ferreira M, Jauniaux T, Llavona A, Öztürk B, Öztürk AA, Ridoux V, Rogan E, Sequeira M, Siebert U, Vikingsson GA, Borrell A, Michaux JR, Aguilar A (*in press*) Postglacial climate changes and rise of three ecotypes of harbour porpoises in western Palearctic waters *Molecular Ecology*.

Fontaine MC, Snirc A, Frantzis A, Koutrakis E, Ozturk B, Ozturk AA, Austerlitz F (2012) History of expansion and anthropogenic collapse in a top marine predator of the Black Sea estimated from genetic data. *Proceedings of the National Academy of Sciences of the United States of America* **109**, E2569-E2576.

Fontaine MC, Tolley KA, Michaux JR, Birkun A, Ferreira M, Jauniaux T, Llavona A, Ozturk B, Ozturk AA, Ridoux V, Rogan E, Sequeira M, Bouquegneau JM, Baird SJE (2010) Genetic and historic evidence for climate-driven population fragmentation in a top cetacean predator: the harbour porpoises in European water. *Proceedings of the Royal Society B-Biological Sciences* **277**, 2829-2837.

Foote AD, Newton J, Avila-Arcos MC, Kampmann ML, Samaniego JA, Post K, Rosing-Asvid A, Sinding MHS, Gilbert MTP (2013) Tracking niche variation over millennial timescales in sympatric killer whale lineages. *Proceedings of the Royal Society B-Biological Sciences* **280**, 20131481.

Foote AD, Newton J, Piertney SB, Willerslev E, Gilbert MTP (2009) Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations. *Molecular Ecology* **18**, 5207-5217.

Foote AD, Vilstrup JT, de Stephanis R, Verborgh P, Nielsen SCA, Deaville R, Kleivane L, Martin V, Miller PJO, Oien N, Perez-Gil M, Rasmussen M, Reid RJ, Robertson KM, Rogan E, Simila T, Tejedor ML, Vester H, Vikingsson GA, Willerslev E, Gilbert MTP, Piertney SB (2011) Genetic differentiation among North Atlantic killer whale populations. *Molecular Ecology* **20**, 629-641.

Forcada J, Gazo M, Aguilar A, Gonzalvo J, Fernandez-Contreras M (2004) Bottlenose dolphin abundance in the NW Mediterranean: addressing heterogeneity in distribution. *Marine Ecology Progress Series* **275**, 275-287.

Foster EA, Franks DW, Morrell LJ, Balcomb KC, Parsons KM, van Ginneken A, Croft DP (2012) Social network correlates of food availability in an endangered population of killer whales, *Orcinus orca*. *Animal Behaviour* **83**, 731-736.

Fraley C, Raftery AE (2002) Model-based clustering, discriminant analysis, and density estimation. *Journal of the American Statistical Association* **97**, 611-631.

Fraley C, Raftery AE, Murphy TB, Scrucca L (2012) mclust version 4 for R: normal mixture modeling for model-based clustering, classification, and density estimation. Technical Report No. 597. Department of Statistics, University of Washington.

Francois O, Durand E (2010) Spatially explicit Bayesian clustering models in population genetics. *Molecular Ecology Resources* **10**, 773-784.

Frederiksen M, Wanless S, Harris MP, Rothery P, Wilson LJ (2004) The role of industrial fisheries and oceanographic change in the decline of North Sea black-legged kittiwakes. *Journal of Applied Ecology* **41**, 1129-1139.

Frère CH, Krützen M, Mann J, Connor RC, Bejder L, Sherwin WB (2010a) Social and genetic interactions drive fitness variation in a free-living dolphin population. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 19949-19954.

Frère CH, Krützen M, Mann J, Watson-Capps JJ, Tsai YJ, Patterson EM, Connor R, Bejder L, Sherwin WB (2010b) Home range overlap, matrilineal and biparental kinship drive female associations in bottlenose dolphins. *Animal Behaviour* **80**, 481-486.

Frey-Roos F, Brodmann PA, Reyer HU (1995) Relationships between food resources, foraging patterns and reproductive success in the water pipit, *Anthus sp. spinoletta*. *Behavioral Ecology* **6**, 287-295.

Fry B (2006) *Stable isotope ecology*, New York.

Funk DJ, Nosil P, Etges WJ (2006) Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 3209-3213.

Funk WC, Blouin MS, Corn PS, Maxell BA, Pilliod DS, Amish S, Allendorf FW (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology* **14**, 483-496.

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution* **27**, 489-496.

Fury CA, Ruckstuhl KE, Harrison PL (2013) Spatial and social sexual segregation patterns in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*). *Plos One* **8**, e52987.

Gaggiotti OE, Bekkevold D, Jorgensen HBH, Foll M, Carvalho GR, Andre C, Ruzzante DE (2009) Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: atlantic herring as a case study. *Evolution* **63**, 2939-2951.

Galindo HM, Olson DB, Palumbi SR (2006) Seascape genetics: A coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology* **16**, 1622-1626.

Game ET, Grantham HS, Hobday AJ, Pressey RL, Lombard AT, Beckley LE, Gjerde K, Bustamante R, Possingham HP, Richardson AJ (2009) Pelagic protected areas: the missing dimension in ocean conservation. *Trends in Ecology & Evolution* **24**, 360-369.

Garroway CJ, Bowman J, Wilson PJ (2013) Complex social structure of southern flying squirrels is related to spatial proximity but not kinship. *Behavioral Ecology and Sociobiology* **67**, 113-122.

Gazda SK, Connor RC, Edgar RK, Cox F (2005) A division of labour with role specialization in group-hunting bottlenose dolphins (*Tursiops truncatus*) off Cedar Key, Florida. *Proceedings of the Royal Society B-Biological Sciences* **272**, 135-140.

Gebremedhin B, Ficetola GF, Naderi S, Rezaei HR, Maudet C, Rioux D, Luikart G, Flagstad O, Thuiller W, Taberlet P (2009) Combining genetic and ecological data to assess the conservation status of the endangered Ethiopian walia ibex. *Animal Conservation* **12**, 89-100.

Geffen E, Waidyaratne S, Dalen L, Angerbjorn A, Vila C, Hersteinsson P, Fuglei E, White PA, Goltsman M, Kapel CMO, Wayne RK (2007) Sea ice occurrence predicts genetic isolation in the Arctic fox. *Molecular Ecology* **16**, 4241-4255.

Gelman A, Carlin J, Stern H, Dunson D, Vehtari A, Rubin D (2003) *Bayesian Data Analysis* CRC Press, London.

Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology* **14**, 1066-1074.

Giménez J, Baron E, Louis M, Verborgh P, Gauffier P, Forero MG, Eljarrat E, Barcelo D, de Stephanis R (2013) A multi-disciplinary approach to define conservation units of bottlenose dolphins (*Tursiops truncatus*). In: *Society for Marine Mammalogy conference*, Dunedin, New Zealand.

Glaubitz JC (2004) CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes* **4**, 309-310.

Gnone G, Bellingeri M, Dhermain F, Dupraz F, Nuti S, Bedocchi D, Moulins A, Rosso M, Alessi J, McCrea RS, Azzellino A, Airoldi S, Portunato N, Laran S, David L, Di Meglio N, Bonelli P, Montesi G, Trucchi R, Fossa F, Wurtz M (2011) Distribution, abundance, and movements of the bottlenose dolphin (*Tursiops truncatus*) in the Pelagos Sanctuary MPA (north-west Mediterranean Sea). *Aquatic Conservation-Marine and Freshwater Ecosystems* **21**, 372-388.

Gobush KS, Mutayoba BM, Wasser SK (2008) Long-term impacts of poaching on relatedness, stress physiology, and reproductive output of adult female African elephants. *Conservation Biology* **22**, 1590-1599.

Godley BJ, Barbosa C, Bruford M, Broderick AC, Catry P, Coyne MS, Formia A, Hays GC, Witt MJ (2010) Unravelling migratory connectivity in marine turtles using multiple methods. *Journal of Applied Ecology* **47**, 769-778.

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate *F*-statistics. *Journal of Heredity* **86**, 485-486.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology* **11**, 1103-1114.

Grabowska-Zhang AM, Wilkin TA, Sheldon BC (2012) Effects of neighbor familiarity on reproductive success in the great tit (*Parus major*). *Behavioral Ecology* **23**, 322-333.

Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's finches. *Science* **296**, 707-711.

Grant PR, Grant BR (2006) Evolution of character displacement in Darwin's finches. *Science* **313**, 224-226.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* **28**, 1140-1162.

Grellier K, Wilson B (2003) Bottlenose dolphins using the Sound of Barra, Scotland. *Aquatic Mammals* **29**, 378–382.

Griffiths SW, Brockmark S, Hojesjo J, Johnsson JI (2004) Coping with divided attention: the advantage of familiarity. *Proceedings of the Royal Society B-Biological Sciences* **271**, 695-699.

Guillot G, Estoup A, Mortier F, Cosson JF (2005) A spatial statistical model for landscape genetics. *Genetics* **170**, 1261-1280.

Guillot G, Leblois R, Coulon A, Frantz AC (2009) Statistical methods in spatial genetics. *Molecular Ecology* **18**, 4734-4756.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696-704.

Guinet C (1991) Intentional stranding apprenticeship and social play in killer whales (*Orcinus orca*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **69**, 2712-2716.

Hall TA (1999) BioEdit: a user-friendy biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-98.

Hamilton WD (1964) The genetical evolution of social behaviour. *Journal of Theoretical Biology* **7**, 1-52.

Hammond PS (1986) Estimating the size of naturally marked whale populations using capture-recapture techniques. *Report of the International Whaling Commission* **Special Issue 8**, 253-282.

Hammond PS, Bearzi G, Bjørge A, Forney KA, Karczmarski L, Kasuya T, Perrin WF, Scott MD, Wang JY, Wells RS, Wilson B (2012) *Tursiops truncatus*. In: *IUCN 2013. IUCN Red List of Threatened Species. Version 2013*.

Hammond PS, Macleod K, Berggren P, Borchers DL, Burt L, Cañadas A, Desportes G, Donovan GP, Gilles A, Gillespie D, Gordon J, Hiby L, Kuklik I, Leaper R, Lehnert K, Leopold M, Lovell P, Øien N, Paxton CGM, Ridoux V, Rogan E, Samarra F, Scheidat M, Sequeira M, Siebert U, Skov H, Swift R, Tasker ML, Teilmann J, Van Canneyt O, Vázquez JA (2013) Cetacean abundance and distribution in European Atlantic shelf waters to inform conservation and management. *Biological Conservation* **64**, 107-122.

Hammond PS, Macleod K, Gillespie D, Swift R, Winship A, Burt ML, Cañadas A, Vázquez JA, Ridoux V, Certain G, Van Canneyt O, Lens S, Santos B, Rogan E, Uriarte A, Hernandez C, Castro R (2009) Cetacean Offshore Distribution and Abundance in the European Atlantic (CODA). Final report to the European Commission. Sea Mammal Research Unit, St Andrews.

Hammond PS, Mizroch SA, Donovan GP (1990) Individual recognition of cetaceans: use of photo-identification and other techniques to estimate population parameters. *Report of the International Whaling Commission* **Special Issue 12**, 440 pp.

Handley LJL, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* **16**, 1559-1578.

Hare MP, Nunney L, Schwartz MK, Ruzzante DE, Burford M, Waples RS, Ruegg K, Palstra F (2011) Understanding and estimating effective population size for practical application in marine species management. *Conservation Biology* **25**, 438-449.

Härkönen TJ (1986) *Guide to the otoliths of the bony fishes of the northeast Atlantic*, Hellerup.

Harlin AD, Markowitz T, Baker CS, Würsig B, Honeycutt RL (2003) Genetic structure, diversity, and historical demography of New Zealand's dusky dolphin (*Lagenorhynchus obscurus*). *Journal of Mammalogy* **84**, 702-717.

Hauver S, Hirsch BT, Prange S, Dubach J, Gehrt SD (2013) Age, but not sex or genetic relatedness, shapes raccoon dominance patterns. *Ethology* **119**, 1-10.

Heithaus MR (2001) Shark attacks on bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia: attack rate, bite scar frequencies and attack seasonality. *Marine Mammal Science* **17**, 526-539.

Helyar SJ, Hemmer-Hansen J, Bekkevold D, Taylor MI, Ogden R, Limborg MT, Cariani A, Maes GE, Diopere E, Carvalho GR, Nielsen EE (2011) Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology Resources* **11**, 123-136.

Hendry AP (2009) Ecological speciation! Or the lack thereof? *Canadian Journal of Fisheries and Aquatic Sciences* **66**, 1383-1398.

Henzi SP, Lusseau D, Weingrill T, van Schaik CP, Barrett L (2009) Cyclicity in the structure of female baboon social networks. *Behavioral Ecology and Sociobiology* **63**, 1015-1021.

Herman DP, Burrows DG, Wade PR, Durban JW, Matkin CO, LeDuc RG, Barrett-Lennard LG, Krahn MM (2005) Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. *Marine Ecology Progress Series* **302**, 275-291.

Hersh SL, Duffield DA (1990) Distinction of Northwestern Atlantic offshore and coastal bottlenose dolphins based on hemoglobin profile and morphometry. In: *The Bottlenose Dolphin* (eds. Leatherwood S, Reeves RR), pp. 129-142. Academic Press, San Diego, California.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-913.

Hey J (2010) Isolation with Migration models for more than two populations. *Molecular Biology and Evolution* **27**, 905-920.

Hicks BD, Staubin DJ, Geraci JR, Brown WR (1985) Epidermal growth in the bottlenose dolphin, *Tursiops truncatus*. *Journal of Investigative Dermatology* **85**, 60-63.

Hijmans RJ, Williams E, Vennes C (2012) geosphere: Spherical trigonometry. R package version 1.2-28. http://CRAN.R-project.org/package=geosphere.

Hill RA, Lee PC (1998) Predation risk as an influence on group size in cercopithecoid primates: implications for social structure. *Journal of Zoology* **245**, 447-456.

Hinde RA (1976) Interactions, relationships and social structure. *Man* **11**, 1-17.

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes 1: Turn-over of ^{13}C in tissues. *Condor* **94**, 181-188.

Hoelzel AR, Dahlheim M, Stern SJ (1998a) Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity* **89**, 121-128.

Hoelzel AR, Hancock JM, Dover GA (1991) Evolution of the cetacean mitochondrial D-Loop region. *Molecular Biology and Evolution* **8**, 475-493.

Hoelzel AR, Hey J, Dahlheim ME, Nicholson C, Burkanov V, Black N (2007) Evolution of population structure in a highly social top predator, the killer whale. *Molecular Biology and Evolution* **24**, 1407-1415.

Hoelzel AR, Potter CW, Best PB (1998b) Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 1177-1183.

Hoffman EA, Kolm N, Berglund A, Arguello JR, Jones AG (2005) Genetic structure in the coral-reef-associated Banggai cardinalfish, *Pterapogon kauderni*. *Molecular Ecology* **14**, 1367-1375.

Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA (2010) Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *Plos Genetics* **6**, e1000862.

Holekamp KE, Cooper SM, Katona CI, Berry NA, Frank LG, Smale L (1997) Patterns of association among female spotted hyenas (*Crocuta crocuta*). *Journal of Mammalogy* **78**, 55-64.

Hollatz C, Flach L, Baker CS, Santos FR (2011) Microsatellite data reveal fine genetic structure in male Guiana dolphins (*Sotalia guianesis*) in two geographically close embayments at south-eastern coast of Brazil. *Marine Biology* **158**, 927-933.

Holm S (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**, 65-70.

Hooker SK, Gerber LR (2004) Marine reserves as a tool for ecosystem-based management: The potential importance of megafauna. *Bioscience* **54**, 27-39.

Huber SK, De Leon LF, Hendry AP, Bermingham E, Podos J (2007) Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proceedings of the Royal Society B-Biological Sciences* **274**, 1709-1714.

Huckstadt LA, Burns JM, Koch PL, McDonald BI, Crocker DE, Costa DP (2012) Diet of a specialist in a changing environment: the crabeater seal along the western Antarctic Peninsula. *Marine Ecology Progress Series* **455**, 287-301.

Hurvich CM, Tsai CL (1989) Regression and time-series model selection in small samples. *Biometrika* **76**, 297-307.

Hutchinson GE (1957) Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology* **22**, 415-427.

Hutchinson GE (1978) *An introduction to population biology* Yale University Press, New Haven.

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* **80**, 595-602.

Jann B, Allen J, Carrillo M, Hanquet S, Katona SK, Martin AR, Reeves RR, Seton R, Stevick PT, Wenzel FW (2003) Migration of a humpback whale (*Megaptera novaeangliae*) between the Cape Verde Islands and Iceland. *Journal of Cetacean Research & Management* **5**, 125-129.

Johansson M, Primmer CR, Merila J (2006) History vs. current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). *Molecular Ecology* **15**, 975-983.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-1405.

Jombart T (2012) A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 1.3-4.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *Bmc Genetics* **11**, 94.

Jorgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. *Molecular Ecology* **14**, 3219-3234.

Karczmarski L, Wursig B, Gailey G, Larson KW, Vanderlip C (2005) Spinner dolphins in a remote Hawaiian atoll: social grouping and population structure. *Behavioral Ecology* **16**, 675-685.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters* **7**, 1225-1241.

Kelley JL, Morrell LJ, Inskip C, Krause J, Croft DP (2011) Predation risk shapes social networks in fission-fusion populations. *Plos One* **6**, e2428010.

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **78**, 1-27.

Kendall WL, Nichols JD, Hines JE (1997) Estimating temporary emigration using capture-recapture data with Pollock's robust design. *Ecology* **78**, 563-578.

Kernaleguen L, Cazelles B, Arnould JPY, Richard P, Guinet C, Cherel Y (2012) Long-term species, sexual and individual variations in foraging strategies of fur seals revealed by stable isotopes in whiskers. *Plos One* **7**, e32916.

King SL, Janik VM (2013) Bottlenose dolphins can use learned vocal labels to address each other. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 13216-13221.

Kingston SE, Adams LD, Rosel PE (2009) Testing mitochondrial sequences and anonymous nuclear markers for phylogeny reconstruction in a rapidly radiating group: molecular systematics of the Delphininae (Cetacea: Odontoceti: Delphinidae). *Bmc Evolutionary Biology* **9**, 245.

Knudsen R, Primicerio R, Amundsen PA, Klemetsen A (2010) Temporal stability of individual feeding specialization may promote speciation. *Journal of Animal Ecology* **79**, 161-168.

Konovalov DA, Manning C, Henshaw MT (2004) KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Molecular Ecology Notes* **4**, 779-782.

Kopps AM, Ackermann CY, Sherwin WB, Allen SJ, Bejder L, Krützen M (2014) Cultural transmission of tool use combined with habitat specializations leads to fine-scale genetic structure in bottlenose dolphins. *Proceedings of the Royal Society B-Biological Sciences* **281**, 20133245.

Krause J, Ruxton GD (2002) *Living in Groups* Oxford University Press, Oxford.

Krützen M, Barre LM, Möller LM, Heithaus MR, Simms C, Sherwin WB (2002) A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops* spp. *Marine Mammal Science* **18**, 863-878.

Krützen M, Mann J, Heithaus MR, Connor RC, Bejder L, Sherwin WB (2005) Cultural transmission of tool use in bottlenose dolphins. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 8939-8943.

Krützen M, Sherwin WB, Connor RC, Barre LM, Van de Casteele T, Mann J, Brooks R (2003) Contrasting relatedness patterns in bottlenose dolphins *(Tursiops* sp.) with different alliance strategies. *Proceedings of the Royal Society B-Biological Sciences* **270**, 497-502.

Kruuk H (1995) *Wild otters: predation and populations* Oxford University Press, Oxford, UK.

Kucklick J, Schwacke L, Wells R, Hohn A, Guichard A, Yordy J, Hansen L, Zolman E, Wilson R, Litz J, Nowacek D, Rowles T, Pugh R, Balmer B, Sinclair C, Rosel P (2011) Bottlenose

dolphins as indicators of persistent organic pollutants in the Western North Atlantic Ocean and Northern Gulf of Mexico. *Environmental Science & Technology* **45**, 4270-4277.

Kuhner MK (2009) Coalescent genealogy samplers: windows into population history. *Trends in Ecology & Evolution* **24**, 86-93.

Labach H, Dhermain F, Dupraz F (2012) Etude de la population des grands dauphins le long des côtes de Provence. Programme de recherche PELAGOS France 2009/2012. Rapport final GIS3M, pp. 1-44.

Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics* **7**, 295-302.

Leatherwood S, Reeves RR (1990) *The Bottlenose Dolphin* Academic Press.

Lebreton JD, Burnham KP, Clobert J, Anderson DR (1992) Modeling survival and testing biological hypotheses using marked animals - a unified approach with case-studies. *Ecological Monographs* **62**, 67-118.

LeDuc RG, Perrin WF, Dizon AE (1999) Phylogenetic relationships among the delphinid cetaceans based on full cytochrome b sequences. *Marine Mammal Science* **15**, 619-648.

Lehmann J, Korstjens AH, Dunbar RIM (2007) Fission-fusion social systems as a strategy for coping with ecological constraints: a primate case. *Evolutionary Ecology* **21**, 613-634.

Lindblad-Toh K, Garber M, Zuk O, Lin MF, Parker BJ, Washietl S, Kheradpour P, Ernst J, Jordan G, Mauceli E, Ward LD, Lowe CB, Holloway AK, Clamp M, Gnerre S, Alfoldi J, Beal K, Chang J, Clawson H, Cuff J, Di Palma F, Fitzgerald S, Flicek P, Guttman M, Hubisz MJ, Jaffe DB, Jungreis I, Kent WJ, Kostka D, Lara M, Martins AL, Massingham T, Moltke I, Raney BJ, Rasmussen MD, Robinson J, Stark A, Vilella AJ, Wen JY, Xie XH, Zody MC, Worley KC, Kovar CL, Muzny DM, Gibbs RA, Warren WC, Mardis ER, Weinstock GM, Wilson RK, Birney E, Margulies EH, Herrero J, Green ED, Haussler D, Siepel A, Goldman N, Pollard KS, Pedersen JS, Lander ES, Kellis M, Broad I, Baylor Coll M, Washington U (2011) A high-resolution map of human evolutionary constraint using 29 mammals. *Nature* **478**, 476-482.

Linklater WL (2000) Adaptive explanation in socio-ecology: lessons from the Equidae. *Biological Reviews* **75**, 1-20.

Liret C (2001) *Domaine vital, utilisation de l'espace et des ressources : les grands dauphins, Tursiops truncatus de l'île de Sein* PhD dissertation, University of Bretagne Occidentale.

Litz JA, Garrison LP, Fieber LA, Martinez A, Contillo JP, Kucklick JR (2007) Fine-scale spatial variation of persistent organic pollutants in bottlenose dolphins (*Tursiops truncatus*) in Biscayne Bay, Florida. *Environmental Science & Technology* **41**, 7222-7228.

Lohmann KJ, Putman NF, Lohmann CMF (2008) Geomagnetic imprinting: a unifying hypothesis of long-distance natal homing in salmon and sea turtles. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 19096-19101.

Lomolino MV, Riddle BR, Brown JH (2006) Biogeography. Sinauer Associates, Sunderland, Massachusetts.

López A (2003) *Estatus dos pequenos cetáceos da plataforma de Galicia.* PhD dissertation, Universidade de Santiago de Compostela.

López A, Pierce GJ, Santos MB, Gracia J, Guerra A (2003) Fishery by-catches of marine mammals in Galician waters: results from on-board observations and an interview survey of fishermen. *Biological Conservation* **111**, 25-40.

Losos JB, Ricklefs RE (2009) Adaptation and diversification on islands. *Nature* **457**, 830-836.

Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology* **19**, 3038-3051.

Lowry DB (2012) Ecotypes and the controversy over stages in the formation of new species. *Biological Journal of the Linnean Society* **106**, 241-257.

Lukacs P (2013) Closed population capture-recapture models. In: *Program Mark - a gentle introduction* (eds. Cooch EG, White GC).

Lusseau D (2003) The emergent properties of a dolphin social network. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S186-S188.

Lusseau D (2007) Why are male social relationships complex in the Doubtful Sound bottlenose dolphin population? *Plos One* **2**, e348.

Lusseau D, Schneider K, Boisseau OJ, Haase P, Slooten E, Dawson SM (2003) The bottlenose dolphin community of Doubtful Sound features a large proportion of long-lasting associations - Can geographic isolation explain this unique trait? *Behavioral Ecology and Sociobiology* **54**, 396-405.

Lusseau D, Williams R, Wilson B, Grellier K, Barton TR, Hammond PS, Thompson PM (2004) Parallel influence of climate on the behaviour of Pacific killer whales and Atlantic bottlenose dolphins. *Ecology Letters* **7**, 1068-1076.

Lusseau D, Wilson B, Hammond PS, Grellier K, Durban JW, Parsons KM, Barton TR, Thompson PM (2006) Quantifying the influence of sociality on population structure in bottlenose dolphins. *Journal of Animal Ecology* **75**, 14-24.

MacAvoy SE, Arneson LS, Bassett E (2006) Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. *Oecologia* **150**, 190-201.

Mace GM, Lande R (1991) Assessing extinction threats - towards a reevaluation of IUCN threatened species categories. *Conservation Biology* **5**, 148-157.

Madsen PT, Wahlberg M, Tougaard J, Lucke K, Tyack P (2006) Wind turbine underwater noise and marine mammals: implications of current knowledge and data needs. *Marine Ecology Progress Series* **309**, 279-295.

Mann J, Sargeant BL (2003) Like mother, like calf: The ontogeny of foraging traditions in wild Indian Ocean bottlenose dolphins (*Tursiops* sp.). In: *The biology of traditions: Models and evidence* (eds. Fragaszy D, Perry S), pp. 236-266. Cambridge University Press.

Mann J, Stanton MA, Patterson EM, Bienenstock EJ, Singh LO (2012) Social networks reveal cultural behaviour in tool-using using dolphins. *Nature Communications* **3**, 980.

Mannocci L, Dabin W, Augeraud-Veron E, Dupuy JF, Barbraud C, Ridoux V (2012) Assessing the impact of bycatch on dolphin populations: the case of the common dolphin in the Eastern North Atlantic. *Plos One* **7**, e32615.

Marcoux M, Whitehead H, Rendell L (2007) Sperm whale feeding variation by location, year, social group and clan: evidence from stable isotopes. *Marine Ecology Progress Series* **333**, 309-314.

Martien KK, Baird RW, Hedrick NM, Gorgone AM, Thieleking JL, McSweeney DJ, Robertson KM, Webster DL (2011) Population structure of island-associated dolphins: Evidence from mitochondrial and microsatellite markers for common bottlenose dolphins (*Tursiops truncatus*) around the main Hawaiian Islands. *Marine Mammal Science* **28**, 208–232.

Martien KK, Taylor BL (2003) Limitations of hypothesis-testing in defining management units for continuously distributed species. *Journal of Cetacean Research & Management* **5**, 213-218.

May SE, Medley KA, Johnson SA, Hoffman EA (2011) Combining genetic structure and ecological niche modeling to establish units of conservation: A case study of an imperiled salamander. *Biological Conservation* **144**, 1441-1450.

Mayr E (1942) Systematics and the origin of species (ed. Press CU), New York.

McComb K, Moss C, Durant SM, Baker L, Sayialel S (2001) Matriarchs as repositories of social knowledge in African elephants. *Science* **292**, 491-494.

Mead JC, Potter CW (1995) Recognizing two populations of the bottlenose dolphin (*Tursiops truncatus*) of the Atlantic coast of North America - morphologic and ecologic considerations. *IBI Reports (International Marine Biological Research Institute, Kamogawa, Japan)* **5**, 31-44.

Mendes S, Newton J, Reid RJ, Zuur AF, Pierce GJ (2007) Stable carbon and nitrogen isotope ratio profiling of sperm whale teeth reveals ontogenetic movements and trophic ecology. *Oecologia* **151**, 605-615.

Méndez-Fernandez P, Webster L, Chouvelon T, Bustamante P, Ferreira M, González AF, López A, Moffat CF, Pierce GJ, Read FL, Russell M, Santos MB, Spitz J, Vingada JV, Caurant F (2014) An assessment of contaminant concentrations in toothed whale species of the NW Iberian Peninsula : Part I. Persistent organic pollutants. *Science of the Total Environment* **484**, 196-205.

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* **142**, 1061-1064.

Milano I, Babbucci M, Cariani A, Atanassova M, Bekkevold D, Carvalho GR, Espineira M, Fiorentino F, Garofalo G, Geffen AJ, Hansen JH, Helyar SJ, Nielsen EE, Ogden R, Patarnello T, Stagioni M, Consortium F, Tinti F, Bargelloni L (2014) Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (*Merluccius merluccius*). *Molecular Ecology* **23**, 118-135.

Milligan GW, Cooper MC (1987) Methodology review - clustering methods. *Applied Psychological Measurement* **11**, 329-354.

Mirimin L, Miller R, Dillane E, Berrow SD, Ingram S, Cross TF, Rogan E (2011) Fine-scale population genetic structuring of bottlenose dolphins in Irish coastal waters. *Animal Conservation* **14**, 342-353.

Mirimin L, Westgate A, Rogan E, Rosel P, Read A, Coughlan J, Cross T (2009) Population structure of short-beaked common dolphins (*Delphinus delphis*) in the North Atlantic Ocean as revealed by mitochondrial and nuclear genetic markers. *Marine Biology* **156**, 821-834.

Möller LM (2011) Sociogenetic structure, kin associations and bonding in delphinids. *Molecular Ecology* **21**, 745-764.

Möller LM, Beheregaray LB, Harcourt RG, Krützen M (2001) Alliance membership and kinship in wild male bottlenose dolphins (*Tursiops aduncus*) of southeastern Australia. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 1941-1947.

Möller LM, Harcourt RG (2008) Shared reproductive state enhances female associations in dolphins. *Research Letters in Ecology* Article ID **498390**.

Möller LM, Valdez FP, Allen S, Bilgmann K, Corrigan S, Beheregaray LB (2011) Fine-scale genetic structure in short-beaked common dolphins (*Delphinus delphis*) along the East Australian Current. *Marine Biology* **158**, 113-126.

Moreno R, Jover L, Munilla I, Velando A, Sanpera C (2010) A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. *Marine Biology* **157**, 545-553.

Moritz C (1994) Defining evolutionarily significant units for conservation. *Trends in Ecology & Evolution* **9**, 373-375.

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology* **51**, 238-254.

Moritz C, Dowling TE, Brown WM (1987) Evolution of animal mitochondrial DNA - relevance for population biology and systematics. *Annual Review of Ecology and Systematics* **18**, 269-292.

Morizur Y, Berrow SD, Tregenza NJC, Couperus AS, Pouvreau S (1999) Incidental catches of marine-mammals in pelagic trawl fisheries of the northeast Atlantic. *Fisheries Research* **41**, 297-307.

Morse DH, Fritz RS (1987) The consequences of foraging for reproductive success. In: *Foraging Behavior* (eds. Kamil AC, Krebs JR, Pulliam HR), pp. 443-455. Plenum Press, New York.

Moura AE, Nielsen SCA, Vilstrup JT, Moreno-Mayar JV, Gilbert MTP, Gray HWI, Natoli A, Mller L, Hoelzel AR (2013) Recent diversification of a marine genus (*Tursiops* spp.) tracks habitat preference and environmental change. *Systematic Biology* **62**, 865-877.

Mourier J, Vercelloni J, Planes S (2012) Evidence of social communities in a spatially structured network of a free-ranging shark species. *Animal Behaviour* **83**, 389-401.

Musiani M, Leonard JA, Cluff HD, Gates C, Mariani S, Paquet PC, Vila C, Wayne RK (2007) Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat colour and association with migratory caribou. *Molecular Ecology* **16**, 4149-4170.

Natoli A, Birkun A, Aguilar A, Lopez A, Hoelzel AR (2005) Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society B-Biological Sciences* **272**, 1217-1226.

Natoli A, Peddemors VM, Hoelzel AR (2004) Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary Biology* **17**, 363-375.

Newman MEJ (2004) Analysis of weighted networks. *Physical Review E* **70**, 056131.

Newsome SD, del Rio CM, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment* **5**, 429-436.

Nichols C, Herman J, Gaggiotti OE, Dobney KM, Parsons K, Hoelzel AR (2007) Genetic isolation of a now extinct population of bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society B-Biological Sciences* **274**, 1611-1616.

Nielsen R (2005) Molecular signatures of natural selection. *Annual Review of Genetics* **39**, 197-218.

Nordborg M (2001) Coalescent theory. In: *Handbook of statistical genetics* (eds. Balding DJ, Bishop M, Cannings C), pp. 213–238. John Wiley, Chichester, United Kingdom.

Nøttestad L, Ferno A, Axelsen BE (2002) Digging in the deep: killer whales' advanced hunting tactic. *Polar Biology* **25**, 939-941.

O'Brien JM, Berrow SD, Ryan C, McGrath D, O'Connor I, Pesante P, Burrows G, Massett N, Klötzer V, Whooley P (2009) A note on long-distance matches of bottlenose dolphins (*Tursiops truncatus*) around the Irish coast using photo-identification. *Journal of Cetacean Research and Management* **11**, 71-76.

Olin JA, Fair PA, Recks MA, Zolman E, Adams J, Fisk AT (2012) Unique seasonal forage bases within a local population of bottlenose dolphin (*Tursiops truncatus*). *Marine Mammal Science* **28**, E28-E40.

Olsen MT, Andersen LW, Dietz R, Teilmann J, Harkonen T, Siegismund HR (2014) Integrating genetic data and population viability analyses for the identification of harbour seal (*Phoca vitulina*) populations and management units. *Molecular Ecology* **23**, 815-831.

Ossi K, Kamilar JM (2006) Environmental and phylogenetic correlates of *Eulemur* behavior and ecology (Primates : Lemuridae). *Behavioral Ecology and Sociobiology* **61**, 53-64.

Otis DL, Burnham KP, White GC, Anderson DR (1978) Statistical inference from capture data on closed animal populations. *Wildlife Monographs* **62**, 7-135.

Owen ECG (2003) *The reproductive and ecological functions of the pair-bond between allied, adult male bottlenose dolphins, Tursiops truncatus, in Sarasota Bay, Florida.* PhD dissertation, University of California.

Owen ECG, Wells RS, Hofmann S (2002) Ranging and association patterns of paired and unpaired adult male Atlantic bottlenose dolphins, *Tursiops truncatus*, in Sarasota, Florida, provide no evidence for alternative male strategies. *Canadian Journal of Zoology* **80**, 2072-2089.

Pace DS, Pulcini M, Triossi F (2012) Anthropogenic food patches and association patterns of *Tursiops truncatus* at Lampedusa island, Italy. *Behavioral Ecology* **23**, 254-264.

Packer C, Ruttan L (1988) The evolution of cooperative hunting. *American Naturalist* **132**, 159-198.

Packer C, Scheel D, Pusey AE (1990) Why lions form groups: food is not enough. *American Naturalist* **136**, 1-19.

Palsbøll PJ, Berube M, Allendorf FW (2007) Identification of management units using population genetic data. *Trends in Ecology & Evolution* **22**, 11-16.

Palstra FP, Ruzzante DE (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology* **17**, 3428-3447.

Pante E, Simon-Bouhet B (2013) marmap: a package for importing, plotting and analyzing bathymetric and topographic data in R. *Plos One* **8**, e73051.

Pardini AT, Jones CS, Noble LR, Kreiser B, Malcolm H, Bruce BD, Stevens JD, Cliff G, Scholl MC, Francis M, Duffy CAJ, Martin AP (2001) Sex-biased dispersal of great white sharks - In some respects, these sharks behave more like whales and dolphins than other fish. *Nature* **412**, 139-140.

Park SDE (2001) *Trypanotolerance in West African cattle and the population genetic effects of selection.* PhD dissertation, University of Dublin.

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *Plos One* **5**, e9672.

Parnell AC, Jackson AL (2011) siar: Stable Isotope Analysis in R. R package version 4.1.3. http://CRAN.R-project.org/package=siar.

Parsons KM, Balcomb KC, Ford JKB, Durban JW (2009) The social dynamics of southern resident killer whales and conservation implications for this endangered population. *Animal Behaviour* **77**, 963-971.

Parsons KM, Durban JW, Claridge DE, Balcomb KC, Noble LR, Thompson PM (2003) Kinship as a basis for alliance formation between male bottlenose dolphins, *Tursiops truncatus*, in the Bahamas. *Animal Behaviour* **66**, 185-194.

Pärt T (2001) Experimental evidence of environmental effects on age-specific reproductive success: the importance of resource quality. *Proceedings of the Royal Society B-Biological Sciences* **268**, 2267-2271.

Pastene LA, Goto M, Kanda N, Zerbini AN, Kerem D, Watanabe K, Bessho Y, Hasegawa M, Nielsen R, Larsen F, Palsboll PJ (2007) Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata*. *Molecular Ecology* **16**, 1481-1495.

Pearse DE, Crandall KA (2004) Beyond F_{ST}: Analysis of population genetic data for conservation. *Conservation Genetics* **5**, 585-602.

Pelletier F, Garant D, Hendry AP (2009) Eco-evolutionary dynamics. *Philosophical Transactions of the Royal Society B-Biological Sciences* **364**, 1483-1489.

Peltier H, Dabin W, Daniel P, Van Canneyt O, Dorémus G, Huon M, Ridoux V (2012) The significance of stranding data as indicators of cetacean populations at sea: Modelling the drift of cetacean carcasses. *Ecological Indicators* **18**, 278–290.

Perrin WF (1984) Patterns of geographical variation in small cetaceans. *Acta Zoologica Fennica* **172**, 137-140.

Perrin WF, Rosel PE, Cipriano F (2013) How to contend with paraphyly in the taxonomy of the delphinine cetaceans? *Marine Mammal Science* **29**, 567-588.

Perrin WF, Thieleking JL, Walker WA, Archer FI, Robertson KM (2011) Common bottlenose dolphins (*Tursiops truncatus*) in California waters: Cranial differentiation of coastal and offshore ecotypes. *Marine Mammal Science* **27**, 769-792.

Pesante G, Evans PGH, Baines ME, McMath M (2008) Abundance and life history parameters of bottlenose dolphin in Cardigan Bay: monitoring 2005-2007. CCW Marine Monitoring Report, pp. 1-77.

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**, 293-320.

Pianka ER (1974) Niche overlap and diffuse competition. *Proceedings of the National Academy of Sciences of the United States of America* **71**, 2141-2145.

Pierce GJ, Boyle PR (1991) A review of methods for diet analysis in piscivorous marine mammals. *Oceanography and Marine Biology* **29**, 409-486.

Piertney SB, MacColl ADC, Bacon PJ, Dallas JF (1998) Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology* **7**, 1645-1654.

Piertney SB, MacColl ADC, Lambin X, Moss R, Dallas JF (1999) Spatial distribution of genetic relatedness in a moorland population of red grouse (*Lagopus lagopus scoticus*). *Biological Journal of the Linnean Society* **68**, 317-331.

Pilot M, Dahlheim ME, Hoelzel AR (2010) Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (*Orcinus orca*). *Journal of Evolutionary Biology* **23**, 20-31.

Pilot M, Jedrzejewski W, Branicki W, Sidorovich VE, Jedrzejewska B, Stachura K, Funk SM (2006) Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology* **15**, 4533-4553.

Pilot M, Jedrzejewski W, Sidorovich VE, Meier-Augenstein W, Hoelzel AR (2012) Dietary differentiation and the evolution of population genetic structure in a highly mobile carnivore. *Plos One* **7**, e39341.

Pinter-Wollman N, Isbell LA, Hart LA (2009) The relationship between social behaviour and habitat familiarity in African elephants (*Loxodonta africana*). *Proceedings of the Royal Society B-Biological Sciences* **276**, 1009-1014.

Pirotta E, Laesser EV, Hardaker A, Riddoch N, Marcoux M, Lusseau D (2013) Dredging displaces bottlenose dolphins from an urbanised foraging patch. *Marine Pollution Bulletin* **14**, 396–402.

Podlesak DW, McWilliams SR, Hatch KA (2005) Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. *Oecologia* **142**, 501-510.

Pollock KH (1982) A capture-recapture design robust to unequal probability of capture. *Journal of Wildlife Management* **46**, 752-757.

Pope TR (1992) The influence of dispersal patterns and mating system on genetic differentiation within and between populations of the red howler monkey (*Alouatta seniculus*). *Evolution* **46**, 1112-1128.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.

Prum RO (1994) Phylogenetic analysis of the evolution of alternative social behavior in the manakins (Aves, Pipridae). *Evolution* **48**, 1657-1675.

Pyke GH, Pulliam HR, Charnov EL (1977) Optimal foraging: a selective review of theory and tests. *The Quarterly Review of Biology* **52**, 137-154.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* **43**, 258-275.

Quérouil S, Silva MA, Freitas L, Prieto R, Magalhaes S, Dinis A, Alves F, Matos JA, Mendonca D, Hammond PS, Santos RS (2007) High gene flow in oceanic bottlenose dolphins (*Tursiops truncatus*) of the North Atlantic. *Conservation Genetics* **8**, 1405-1419.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramos-Fernandez G, Boyer D, Gomez VP (2006) A complex social structure with fission-fusion properties can emerge from a simple foraging model. *Behavioral Ecology and Sociobiology* **60**, 536-549.

Ramos R, Gonzalez-Solis J (2012) Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. *Frontiers in Ecology and the Environment* **10**, 258-266.

Raxworthy CJ, Ingram CM, Rabibisoa N, Pearson RG (2007) Applications of ecological niche modeling for species delimitation: A review and empirical evaluation using day geckos (*Phelsuma*) from Madagascar. *Systematic Biology* **56**, 907-923.

Raymond M, Rousset F (1995) GENEPOP (version 1.2) - Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Rayner MJ, Hauber ME, Steeves TE, Lawrence HA, Thompson DR, Sagar PM, Bury SJ, Landers TJ, Phillips RA, Ranjard L, Shaffer SA (2011) Contemporary and historical separation of transequatorial migration between genetically distinct seabird populations. *Nature Communications* **2**, 332.

Read AJ, Urian KW, Wilson B, Waples DM (2003) Abundance of bottlenose dolphins in the bays, sounds, and estuaries of North Carolina. *Marine Mammal Science* **19**, 59-73.

Reijinders PJH, Aguilar A (2002) Pollution and marine mammals. In: *Encyclopedia of Marine Mammals* (eds. Perrin WF, Würsig B, Thewissen JGM), pp. 948–956. Academic Press, San Diego, California.

Rey O, Estoup A, Vonshak M, Loiseau A, Blanchet S, Calcaterra L, Chifflet L, Rossi JP, Kergoat GJ, Foucaud J, Orivel J, Leponce M, Schultz T, Facon B (2012) Where do adaptive shifts occur

during invasion? A multidisciplinary approach to unravelling cold adaptation in a tropical ant species invading the Mediterranean area. *Ecology Letters* **15**, 1266-1275.

Riesch R, Barrett-Lennard LG, Ellis GM, Ford JKB, Deecke VB (2012) Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? *Biological Journal of the Linnean Society* **106**, 1-17.

Rioux E, Lesage V, Postma L, Pelletier E, Turgeon J, Stewart REA, Stern G, Hammill MO (2012) Use of stable isotopes and trace elements to determine harvest composition and wintering assemblages of belugas at a contemporary ecological scale. *Endangered Species Research* **18**, 179–191.

Robineau D (2005) *Faune de France 89. Cétacés de France.*

Robinson JD, Moyer GR (2013) Linkage disequilibrium and effective population size when generations overlap. *Evolutionary Applications* **6**, 290-302.

Robinson KP, O'Brien J, Berrow SD, Cheney B, Costa M, Eisfeld SM, Haberline D, Mandlebreg L, O'Donovan M, Machiel G, Oudejans MG, Ryan C, Stevick PT, Thompson PM, Whooley P (2012) Discrete or not so discrete: Long distance movements by coastal bottlenose dolphins in UK and Irish waters. *Journal of Cetacean Research & Management* **12**, 365-371.

Rock J, Pastene LA, Kaufman GD, Forestell P, Matsuoka K, Allen J (2006) A note on East Australia Group V Stock humpback whale movement between feeding and breeding areas based on photo-identification. *Journal of Cetacean Research & Management* **8**, 301-305.

Rode KD, Amstrup SC, Regehr EV (2010) Reduced body size and cub recruitment in polar bears associated with sea ice decline. *Ecological Applications* **20**, 768-782.

Rogan E, Mackey M (2007) Megafauna bycatch in drift nets for albacore tuna (*Thunnus alalunga*) in the NE Atlantic. *Fisheries Research* **86**, 6-14.

Rohling EJ, Abu-Zied R, Casford CSL, Hayes A, Hoogakker BAA (2009) The Mediterranean Sea: present and past. In: *Physical geography of the Mediterranean Basin* (ed. Woodward JC), pp. 33-67. Oxford University Press, Oxford, UK.

Rohling EJ, Dendulk M, Pujol C, Vergnaudgrazzini C (1995) Abrupt hydrographic change in the Alboran Sea (Western Mediterranean) around 8000 yrs BP. *Deep-Sea Research Part I-Oceanographic Research Papers* **42**, 1609-1619.

Rooker JR, Secor DH, De Metrio G, Schloesser R, Block BA, Neilson JD (2008a) Natal homing and connectivity in Atlantic bluefin tuna populations. *Science* **322**, 742-744.

Rooker JR, Secor DH, DeMetrio G, Kaufman AJ, Rios AB, Ticina V (2008b) Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths. *Marine Ecology Progress Series* **368**, 231-239.

Rosel PE (2003) PCR-based sex determination in Odontocete cetaceans. *Conservation Genetics* **4**, 647-649.

Rosel PE, Hansen L, Hohn AA (2009) Restricted dispersal in a continuously distributed marine species: common bottlenose dolphins *Tursiops truncatus* in coastal waters of the western North Atlantic. *Molecular Ecology* **18**, 5030-5045.

Rossiter SJ, Zubaid A, Mohd-Adnan A, Struebig MJ, Kunz TH, Gopal S, Petit EJ, Kingston T (2012) Social organization and genetic structure: insights from codistributed bat populations. *Molecular Ecology* **21**, 647-661.

Roughgarden J (1972) Evolution of niche width. *American Naturalist* **106**, 683-718.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.

Rozas J, Rozas R (1999) DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* **15**, 174-175.

Rubenstein DI, Wrangham RW (1986) *Ecological aspects of social evolution: birds and mammals* Princeton University Press, Princeton.

Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology & Evolution* **19**, 256-263.

Ruckstuhl KE (2007) Sexual segregation in vertebrates: proximate and ultimate causes. *Integrative and Comparative Biology* **47**, 245-257.

Rueness EK, Stenseth NC, O'Donoghue M, Boutin S, Ellegren H, Jakobsen KS (2003) Ecological and genetic spatial structuring in the Canadian lynx. *Nature* **425**, 69-72.

Rundle HD, Nagel L, Boughman JW, Schluter D (2000) Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**, 306-308.

Rundle HD, Nosil P (2005) Ecological speciation. *Ecology Letters* **8**, 336-352.

Russello MA, Kirk SL, Frazer KK, Askey PJ (2012) Detection of outlier loci and their utility for fisheries management. *Evolutionary Applications* **5**, 39-52.

Rutledge LY, Patterson BR, Mills KJ, Loveless KM, Murray DL, White BN (2010) Protection from harvesting restores the natural social structure of eastern wolf packs. *Biological Conservation* **143**, 332-339.

Sacks BN, Bannasch DL, Chomel BB, Ernest HB (2008) Coyotes demonstrate how habitat specialization by individuals of a generalist species can diversify populations in a heterogeneous ecoregion. *Molecular Biology and Evolution* **25**, 1384-1394.

Sacks BN, Brown SK, Ernest HB (2004) Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. *Molecular Ecology* **13**, 1265-1275.

Sacks BN, Mitchell BR, Williams CL, Ernest HB (2005) Coyote movements and social structure along a cryptic population genetic subdivision. *Molecular Ecology* **14**, 1241-1249.

Safi K, Kerth G (2007) Comparative analyses suggest that information transfer promoted sociality in male bats in the temperate zone. *American Naturalist* **170**, 465-472.

Santos MB, Clarke MR, Pierce GJ (2001a) Assessing the importance of cephalopods in the diets of marine mammals and other top predators: problems and solutions. *Fisheries Research* **52**, 121-139.

Santos MB, Pierce GJ, Boyle PR, Reid RJ, Ross HM, Patterson IAP, Kinze CC, Tougaard S, Lick R, Piatkowski U, Hernandez-Garcia V (1999) Stomach contents of sperm whales *Physeter macrocephalus* stranded in the North Sea 1990-1996. *Marine Ecology Progress Series* **183**, 281-294.

Santos MB, Pierce GJ, Reid RJ, Patterson IAP, Ross HM, Mente E (2001b) Stomach contents of bottlenose dolphins (*Tursiops truncatus*) in Scottish waters. *Journal of the Marine Biological Association of the United Kingdom* **81**, 873-878.

Sargeant BL, Mann J (2009) Developmental evidence for foraging traditions in wild bottlenose dolphins. *Animal Behaviour* **78**, 715-721.

Sargeant BL, Wirsing AJ, Heithaus MR, Mann J (2007) Can environmental heterogeneity explain individual foraging variation in wild bottlenose dolphins (*Tursiops* sp.)? *Behavioral Ecology and Sociobiology* **61**, 679-688.

Savolainen O, Lascoux M, Merila J (2013) Ecological genomics of local adaptation. *Nature Reviews Genetics* **14**, 807-820.

Schluter D (2001) Ecology and the origin of species. *Trends in Ecology & Evolution* **16**, 372-380.

Schoener TW (1971) Theory of feeding strategies. *Annual Review of Ecology and Systematics* **2**, 369-404

Schwacke LH, Voit EO, Hansen LJ, Wells RS, Mitchum GB, Hohn AA, Fair PA (2002) Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the southeast United States coast. *Environmental Toxicology and Chemistry* **21**, 2752-2764.

Schwacke LH, Zolman ES, Balmer BC, De Guise S, George RC, Hoguet J, Hohn AA, Kucklick JR, Lamb S, Levin M, Litz JA, McFee WE, Place NJ, Townsend FI, Wells RS, Rowles TK (2012) Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society B-Biological Sciences* **279**, 48-57.

Schwarz CJ, Seber GA (1999) Estimating animal abundance: review III. *Statistical Science* **14**, 427-456.

Segura I, Rocha-Olivares A, Flores-Ramirez S, Rojas-Bracho L (2006) Conservation implications of the genetic and ecological distinction of *Tursiops truncatus* ecotypes in the Gulf of California. *Biological Conservation* **133**, 336-346.

Selkoe KA, Watson JR, White C, Ben Horin T, Iacchei M, Mitarai S, Siegel DA, Gaines SD, Toonen RJ (2010) Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology* **19**, 3708-3726.

Sellas AB, Wells RS, Rosel PE (2005) Mitochondrial and nuclear DNA analyses reveal fine scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico. *Conservation Genetics* **6**, 715-728.

Serrouya R, Paetkau D, McLellan BN, Boutin S, Campbell M, Jenkins DA (2012) Population size and major valleys explain microsatellite variation better than taxonomic units for caribou in western Canada. *Molecular Ecology* **21**, 2588-2601.

Shikano T, Shimada Y, Herczeg G, Merila J (2010) History vs. habitat type: explaining the genetic structure of European nine-spined stickleback (*Pungitius pungitius*) populations. *Molecular Ecology* **19**, 1147-1161.

Silk JB (2007) Social components of fitness in primate groups. *Science* **317**, 1347-1351.

Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL (2010) Strong and consistent social bonds enhance the longevity of female baboons. *Current Biology* **20**, 1359-1361.

Silva MA (2007) *Population biology of bottlenose dolphins in the Azores archipelago.* PhD dissertation, University of St Andrews.

Silva MA, Prieto R, Magalhaes S, Seabra M, Santos R, Hammond P (2008) Ranging patterns of bottlenose dolphins living in oceanic waters: implications for population structure. *Marine Biology* **156**, 179-192.

Siwertsson A, Knudsen R, Praebel K, Adams CE, Newton J, Amundsen PA (2013) Discrete foraging niches promote ecological, phenotypic, and genetic divergence in sympatric whitefish (*Coregonus lavaretus*). *Evolutionary Ecology* **27**, 547-564.

Skrbinsek T, Jelencic M, Waits L, Kos I, Jerina K, Trontelj P (2012) Monitoring the effective population size of a brown bear (*Ursus arctos*) population using new single-sample approaches. *Molecular Ecology* **21**, 862-875.

Skúlason S, Smith TB (1995) Resource polymorphisms in vertebrates. *Trends in Ecology & Evolution* **10**, 366-370.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264-279.

Smith JE, Kolowski JM, Graham KE, Dawes SE, Holekamp KE (2008) Social and ecological determinants of fission-fusion dynamics in the spotted hyaena. *Animal Behaviour* **76**, 619-636.

Smith TB, Skúlason S (1996) Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annual Review of Ecology and Systematics* **27**, 111-133.

Smolker RA, Richards AF, Connor RC, Pepper JW (1992) Sex-differences in patterns of association among Indian Ocean bottlenose dolphins. *Behaviour* **123**, 38-69.

Spitz J, Rousseau Y, Ridoux V (2006) Diet overlap between harbour porpoise and bottlenose dolphin: An argument in favour of interference competition for food? *Estuarine Coastal and Shelf Science* **70**, 259-270.

Spitz J, Trites AW, Becquet V, Brind'Amour A, Cherel Y, Galois R, Ridoux V (2012) Cost of living dictates what whales, dolphins and porpoises eat: the importance of prey quality on predator foraging strategies. *Plos One* **7**, e50096.

Stanley TR, Burnham KP (1999) A closure test for time-specific capture-recapture data. *Environmental and Ecological Statistics* **6**, 197-209.

Stanton MA, Gibson QA, Mann J (2011) When mum's away: a study of mother and calf ego networks during separations in wild bottlenose dolphins (*Tursiops* sp.). *Animal Behaviour* **82**, 405-412.

Stapley J, Reger J, Feulner PGD, Smadja C, Galindo J, Ekblom R, Bennison C, Ball AD, Beckerman AP, Slate J (2010) Adaptation genomics: the next generation. *Trends in Ecology & Evolution* **25**, 705-712.

Steckenreuter A, Möller LM, Harcourt R (2012) How does Australia's largest dolphin-watching industry affect the behaviour of a small and resident population of Indo-Pacific bottlenose dolphins? *Journal of Environmental Management* **97**, 14-21.

Storz JF (1999) Genetic consequences of mammalian social structure. *Journal of Mammalogy* **80**, 553-569.

Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology* **14**, 671-688.

Sugg DW, Chesser RK, Dobson FS, Hoogland JL (1996) Population genetics meets behavioral ecology. *Trends in Ecology & Evolution* **11**, 338-342.

Sundaresan SR, Fischhoff IR, Dushoff J, Rubenstein DI (2007) Network metrics reveal differences in social organization between two fission-fusion species, Grevy's zebra and onager. *Oecologia* **151**, 140-149.

Sutherland WJ (1998) The importance of behavioural studies in conservation biology. *Animal Behaviour* **56**, 801-809.

Tallmon DA, Gregovich D, Waples RS, Baker CS, Jackson J, Taylor BL, Archer E, Martien KK, Allendorf FW, Schwartz MK (2010) When are genetic methods useful for estimating contemporary abundance and detecting population trends? *Molecular Ecology Resources* **10**, 684-692.

Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008) ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. *Molecular Ecology Resources* **8**, 299-301.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512-526.

Taylor BL, Chivers SJ, Larese J, Perrin WF (2007) Generation length and percent mature estimates for IUCN assessments of cetaceans. Administrative Report LJ-07-01 National Marine Fisheries Service, Southwest Fisheries Science Center.

Taylor BL, Dizon AE (1999) First policy then science: why a management unit based solely on genetic criteria cannot work. *Molecular Ecology* **8**, S11-S16.

Taylor EB, McPhail JD (2000) Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proceedings of the Royal Society B-Biological Sciences* **267**, 2375-2384.

Teilmann J, Carstensen J (2012) Negative long term effects on harbour porpoises from a large scale offshore wind farm in the Baltic-evidence of slow recovery. *Environmental Research Letters* **7**.

Tezanos-Pinto G (2009) *Population structure, abundance and reproductive parameters of bottlenose dolphins (Tursiops truncatus) in the Bay of Islands (Northland, New Zealand).* PhD dissertation, University of Auckland.

Tezanos-Pinto G, Baker CS (2011) Short-term reactions and long-term responses of bottlenose dolphins (*Tursiops truncatus*) to remote biopsy sampling. *New Zealand Journal of Marine and Freshwater Research* **46**, 13-29.

Tezanos-Pinto G, Baker CS, Russell K, Martien K, Baird RW, Hutt A, Stone G, Mignucci-Giannoni AA, Caballero S, Endo T, Lavery S, Oremus M, Olavarria C, Garrigue C (2009) A worldwide perspective on the population structure and genetic diversity of bottlenose dolphins (*Tursiops truncatus*) in New Zealand. *Journal of Heredity* **100**, 11-24.

Thayer JA, Sydeman WJ (2007) Spatio-temporal variability in prey harvest and reproductive ecology of a piscivorous seabird, *Cerorhinca monocerata*, in an upwelling system. *Marine Ecology Progress Series* **329**, 253-265.

Thibert-Plante X, Hendry AP (2010) When can ecological speciation be detected with neutral loci? *Molecular Ecology* **19**, 2301-2314.

Thorpe RS, Reardon JT, Malhotra A (2005) Common garden and natural selection experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*). *American Naturalist* **165**, 495-504.

Thorrold SR, Latkoczy C, Swart PK, Jones CM (2001) Natal homing in a marine fish metapopulation. *Science* **291**, 297-299.

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for delta ^{13}C analysis of diet. *Oecologia* **57**, 32-37.

Tixier P (2012) *Déprédation par les orques (Orcinus orca) et les cachalots (Physeter macrocephalus) sur les palangriers à la légine australe dans la ZEE de l'archipel de Crozet.* PhD dissertation, Université Aix-Marseille II.

Torres LG, Read AJ (2009) Where to catch a fish? The influence of foraging tactics on the ecology of bottlenose dolphins (*Tursiops truncatus*) in Florida Bay, Florida. *Marine Mammal Science* **25**, 797-815.

Tougaard J, Carstensen J, Teilmann J, Skov H, Rasmussen P (2009) Pile driving zone of responsiveness extends beyond 20 km for harbor porpoises (*Phocoena phocoena* (L.)). *Journal of the Acoustical Society of America* **126**, 11-14.

Trivers RL (1972) Parental investment and sexual selection. In: *Sexual selection and the sescent of man 1871-1971* (ed. Campbell B), pp. 136-179, Aldine, Chicago.

Tsai YJJ, Mann J (2013) Dispersal, philopatry, and the role of fission-fusion dynamics in bottlenose dolphins. *Marine Mammal Science* **29**, 261-279.

Turesson G (1922a) The genotypical response of the plant species to the habitat. *Hereditas* **3**, 211-350.

Turesson G (1922b) The species and the variety as ecological units. *Hereditas* **3**, 100-113.

Tyne JA, Loneragan NR, Kopps AM, Allen SJ, Krützen M, Bejder L (2012) Ecological characteristics contribute to sponge distribution and tool use in bottlenose dolphins *Tursiops* sp. *Marine Ecology Progress Series* **444**, 143-153.

Ujvari B, Dowton M, Madsen T (2007) Mitochondrial DNA recombination in a free-ranging Australian lizard. *Biology Letters* **3**, 189-192.

Valenzuela LO, Sironi M, Rowntree VJ, Seger J (2009) Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*). *Molecular Ecology* **18**, 782-791.

Valsecchi E, Hale P, Corkeron P, Amos W (2002) Social structure in migrating humpback whales (*Megaptera novaeangliae*). *Molecular Ecology* **11**, 507-518.

Van Bree PJH (1971) On *Globicephala sieboldii* Gray, 1846, and other species of pilot whales (Notes on Cetacea, Delphinoidea III). *Beaufortia* **19**, 79 – 87.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

van Schaik CP (1989) The ecology of social relationships amongst female primates. In: *Comparative Socioecology* (eds. Standen V, Foley RA), pp. 195-218. Blackwell, Oxford.

Venables WN, Ripley BD (2002) Modern Applied Statistics with S (ed. Edition F). Springer, New York.

Verborgh P, de Stephanis R, Perez S, Jaget Y, Barbraud C, Guinet C (2009) Survival rate, abundance, and residency of long-finned pilot whales in the Strait of Gibraltar. *Marine Mammal Science* **25**, 523-536.

Verdu P, Austerlitz F, Estoup A, Vitalis R, Georges M, Thery S, Froment A, Le Bomin S, Gessain A, Hombert JM, Van der Veen L, Quintana-Murci L, Bahuchet S, Heyer E (2009) Origins and genetic diversity of pygmy hunter-gatherers from Western Central Africa. *Current Biology* **19**, 312-318.

Viaud-Martinez KA, Brownell RL, Komnenou A, Bohonak AJ (2008) Genetic isolation and morphological divergence of Black Sea bottlenose dolphins. *Biological Conservation* **141**, 1600-1611.

Viaud-Martinez KA, Vergara MM, Gol'din PE, Ridoux V, Ozturk AA, Ozturk B, Rosel PE, Frantzis A, Komnenou A, Bohonak AJ (2007) Morphological and genetic differentiation of the Black Sea harbour porpoise *Phocoena phocoena*. *Marine Ecology Progress Series* **338**, 281-294.

Vollmer NL, Viricel A, Wilcox L, Moore MK, Rosel PE (2011) The occurrence of mtDNA heteroplasmy in multiple cetacean species. *Current Genetics* **57**, 115-131.

Votier SC, Hatchwell BJ, Beckerman A, McCleery RH, Hunter FM, Pellatt J, Trinder M, Birkhead TR (2005) Oil pollution and climate have wide-scale impacts on seabird demographics. *Ecology Letters* **8**, 1157-1164.

Vucetich JA, Peterson RO, Waite TA (1997) Effects of social structure and prey dynamics on extinction risk in gray wolves. *Conservation Biology* **11**, 957-965.

Walker JL, Potter CW, Macko SA (1999) The diets of modern and historic bottlenose dolphin populations reflected through stable isotopes. *Marine Mammal Science* **15**, 335-350.

Walker WA (1981) Geographic variation in morphology and biology of bottlenose dolphins (*Tursiops*) in the eastern North Pacific. NOAA/NMFS Administrative Report LJ-81-0003c.

Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB, Chaloupka MY, Hutchinson BJ, Abreu-Grobois FA, Amorocho D, Bjorndal KA, Bourjea J, Bowen BW, Duenas RB, Casale P, Choudhury BC, Costa A, Dutton PH, Fallabrino A, Girard A, Girondot M, Godfrey MH, Hamann M, Lopez-Mendilaharsu M, Marcovaldi MA, Mortimer JA, Musick JA, Nel R, Pilcher NJ, Seminoff JA, Troeng S, Witherington B, Mast RB (2010) Regional Management Units for marine turtles: a novel framework for prioritizing conservation and research across multiple scales. *Plos One* **5**, e15465.

Wang JY, Chou LS, White BN (1999) Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus : *Tursiops*) in Chinese waters. *Molecular Ecology* **8**, 1603-1612.

Wang JY, Chou LS, White BN (2000a) Differences in the external morphology of two sympatric species of bottlenose dolphins (genus *Tursiops*) in the waters of China. *Journal of Mammalogy* **81**, 1157-1165.

Wang JY, Chou LS, White BN (2000b) Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters. *Journal of Zoology* **252**, 147-162.

Waples RS (2005) Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? *Molecular Ecology* **14**, 3335-3352.

Waples RS (2010) Spatial-temporal stratifications in natural populations and how they affect understanding and estimation of effective population size. *Molecular Ecology Resources* **10**, 785-796.

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* **8**, 753-756.

Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* **3**, 244-262.

Waples RS, England PR (2011) Estimating contemporary effective population size on the basis of linkage disequilibrium in the face of migration. *Genetics* **189**, 633-644.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* **15**, 1419-1439.

Waples RS, Punt AE, Cope JM (2008) Integrating genetic data into management of marine resources: how can we do it better? *Fish and Fisheries* **9**, 423-449.

Watt CA, Heide-Jørgensen MP, Ferguson SH (2013) How adaptable are narwhal? A comparison of foraging patterns among the world's three narwhal populations. *Ecosphere* **4**, art71.

Weinstein TAR, Capitanio JP (2008) Individual differences in infant temperament predict social relationships of yearling rhesus monkeys, *Macaca mulatta*. *Animal Behaviour* **76**, 455-465.

Weller DW, Cockcroft VG, Würsig B, Lynn SK, Fertl D (1997) Behavioral responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing. *Aquatic Mammals* **23**, 49-58.

Wells RS (1991) The role of long-term study in understanding the social structure of a bottlenose dolphin community. In: *Dolphin societies : Discoveries and Puzzles* (eds. Pryor K, Kenneth SN), pp. 198-225. University of California Press, Ltd., Berkeley.

Wells RS, Rhinehart HL, Cunningham P, Whaley J, Baran M, Koberna C, Costa DP (1999) Long distance offshore movements of bottlenose dolphins. *Marine Mammal Science* **15**, 1098-1114.

Wells RS, Scott MD (1999) Bottlenose dolphin *Tursiops truncatus* (Montagu, 1821). In: *Handbook of Marine Mammals* (eds. Ridgway SH, Harrison R), pp. 137-182. Academic Press, San Diego.

Wells RS, Scott MD, Irvine AB (1987) The social structure of free-ranging bottlenose dolphins. In: *Current mammalogy* (ed. Genoways HH), pp. 247-305. Plenum Press, New York and London.

Wey TW, Blumstein DT (2010) Social cohesion in yellow-bellied marmots is established through age and kin structuring. *Animal Behaviour* **79**, 1343-1352.

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study* **46**, 120-139.

Whitehead H (1995) Investigating structure and temporal scale in social organizations using identified individuals. *Behavioral Ecology* **6**, 199-208.

Whitehead H (1998) Cultural selection and genetic diversity in matrilineal whales. *Science* **282**, 1708-1711.

Whitehead H (1999) Testing association patterns of social animals. *Animal Behaviour* **57**, 26-29.

Whitehead H (2007) Selection of models of lagged identification rates and lagged association rates using AIC and QAIC. *Communications in Statistics-Simulation and Computation* **36**, 1233-1246.

Whitehead H (2008a) *Analyzing animal societies: quantitative methods for vertebrate social analysis* University of Chicago Press, Chicago.

Whitehead H (2008b) Precision and power in the analysis of social structure using associations. *Animal Behaviour* **75**, 1093-1099.

Whitehead H (2009a) Programs for analyzing social structure. SOCPROG2.4 (for MATLAB7.7.0.471, release 2008b).

Whitehead H (2009b) SOCPROG programs: analysing animal social structures. *Behavioral Ecology and Sociobiology* **63**, 765-778.

Whitehead H, Dufault S (1999) Techniques for analyzing vertebrate social structure using identified individuals: review and recommendations. *Advances in the Study of Behavior* **28**, 33-74.

Whitehead H, Gowans S, Faucher A, McCarrey SW (1997) Population analysis of northern bottlenose whales in the Gully, Nova Scotia. *Marine Mammal Science* **13**, 173-185.

Whitehead H, Rendell L (2004) Movements, habitat use and feeding success of cultural clans of South Pacific sperm whales. *Journal of Animal Ecology* **73**, 190-196.

Whitehead H, Rendell L, Osborne RW, Würsig B (2004) Culture and conservation of non-humans with reference to whales and dolphins: review and new directions. *Biological Conservation* **120**, 427-437.

Wierucka K, Verborgh P, Meade R, Colmant L, Gauffier P, Esteban R, de Stephanis R, Cañadas A (2014) Effects of a morbillivirus epizootic on long-finned pilot whales *Globicephala melas* in Spanish Mediterranean waters. *Marine Ecology Progress Series* **502**, 1-10.

Williams R, Lusseau D (2006) A killer whale social network is vulnerable to targeted removals. *Biology Letters* **2**, 497-500.

Wilson B (1995) *The ecology of bottlenose dolphins in the Moray Firth, Scotland: a population at the northern extreme of the species' range.* PhD dissertation, University of Aberdeen.

Wilson B, Hammond PS, Thompson PM (1999) Estimating size and assessing trends in a coastal bottlenose dolphin population. *Ecological Applications* **9**, 288-300.

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**, 1177-1191.

Wilson RM, Kucklick JR, Balmer BC, Wells RS, Chanton JP, Nowacek DP (2012) Spatial distribution of bottlenose dolphins (*Tursiops truncatus*) inferred from stable isotopes and priority organic pollutants. *Science of the Total Environment* **425**, 223-230.

Wiszniewski J, Allen SJ, Möller LM (2009) Social cohesion in a hierarchically structured embayment population of Indo-Pacific bottlenose dolphins. *Animal Behaviour* **77**, 1449-1457.

Wiszniewski J, Beheregaray LB, Allen SJ, Möller L (2010a) Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia. *Conservation Genetics* **11**, 1405-1419.

Wiszniewski J, Brown C, Möller LM (2012a) Complex patterns of male alliance formation in a dolphin social network. *Journal of Mammalogy* **93**, 239–250.

Wiszniewski J, Corrigan S, Beheregaray LB, Möller LM (2012b) Male reproductive success increases with alliance size in Indo-Pacific bottlenose dolphins. *Journal of Animal Ecology* **81**, 423-431.

Wiszniewski J, Lusseau D, Möller LM (2010b) Female bisexual kinship ties maintain social cohesion in a dolphin network. *Animal Behaviour* **80**, 895-904.

Wittemyer G, Douglas-Hamilton I, Getz WM (2005) The socioecology of elephants: analysis of the processes creating multitiered social structures. *Animal Behaviour* **69**, 1357-1371.

Witteveen BH, Worthy GAJ, Wynne KM, Roth JD (2009) Population structure of North Pacific humpback whales on their feeding grounds revealed by stable carbon and nitrogen isotope ratios. *Marine Ecology Progress Series* **379**, 299-310.

Woillez M, Poulard JC, Rivoirard J, Petitgas P, Bez N (2007) Indices for capturing spatial patterns and their evolution in time, with application to European hake (*Merluccius merluccius*) in the Bay of Biscay. *Ices Journal of Marine Science* **64**, 537-550.

Wolf JBW, Harrod C, Brunner S, Salazar S, Trillmich F, Tautz D (2008) Tracing early stages of species differentiation: Ecological, morphological and genetic divergence of Galapagos sea lion populations. *Bmc Evolutionary Biology* **8**, 150.

Worton BJ (1989) Kernel methods for estimating the utilization distribution in home-range studies. *Ecology* **70**, 164-168.

Wrangham RW (1980) An ecological model of female-bonded primate groups. *Behaviour* **75**, 262-300.

Wrangham RW, Gittleman JL, Chapman CA (1993) Constraints on group-size in primates and carnivores: population-density and day-range as assays of exploitation competition. *Behavioral Ecology and Sociobiology* **32**, 199-209.

Wright S (1951) The genetical structure of populations. *Annals of Human Genetics* **15**, 323–354.

Wrona FJ, Dixon RWJ (1991) Group size and predation risk: a field analysis of encounter and dilution effects. *American Naturalist* **137**, 186-201.

Würsig B, Jefferson RA (1990) Methods of photo-identification for small cetaceans. *Reports of the International Whaling Commission* **Special Issue 12**, 43-52.

Würsig B, Würsig M (1977) Photographic determination of group size, composition and stability of coastal porpoises (*Tursiops truncatus*). *Science* **198**, 755-756.

Xavier JC, Cherel Y (2009) *Cephalopod beak guide for the Southern Ocean*, Cambridge UK.

Yoder JB, Clancey E, Des Roches S, Eastman JM, Gentry L, Godsoe W, Hagey TJ, Jochimsen D, Oswald BP, Robertson J, Sarver BAJ, Schenk JJ, Spear SF, Harmon LJ (2010) Ecological

opportunity and the origin of adaptive radiations. *Journal of Evolutionary Biology* **23**, 1581-1596.

Zouros E, Ball AO, Saavedra C, Freeman KR (1994) An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 7463-7467.

APPENDIX

1) Appendix Chapter 4

Appendix A4.1. PCR and genotyping conditions for each microsatellite locus.

Samples were genotyped at 27 microsatellite loci including 21 published markers: EV37 (Valsecchi & Amos 1996), KMW12a (Hoelzel *et al.* 1998), MK5, MK6, MK8, MK9 (Krützen *et al.* 2001), TexVet 5, TexVet 7 (Rooney *et al.* 1999), Ttr04, Ttr11, Ttr34, Ttr48, Ttr58, Ttr63, TtrFF6, TtRH1 (Rosel *et al.* 2005), Tur4_87, Tur4_98, Tur4_128 and Tur4_142 (Nater *et al.* 2009) and 6 markers newly developed during this study: Tut01, Tut02, Tut05, Tut08, Tut09 and Tut10 (see methodology in Appendix A4.2; GENBANK accession numbers are respectively KF887998 to KF888002 for Tut01 to Tut09; all markers are detailed in the below table). Two markers (TtrRH1 and Tut10) were excluded from the analyses because of amplification issues (null alleles or stuttering). Genotyping was performed on a LICOR 4300 DNA analyzer (Sciencetec) for 18 loci and on a 3730XL ABI DNA sequencer (Applied Biosystems) for 7 loci. All the individuals were screened for a particular locus using the same analyzer. For loci analyzed on the LICOR 4300 sequencer, each 10 µL PCR reaction contained 1 µl of extracted DNA, 1X reaction Buffer, 0.25 mM dNTPs, 1.5 mM MgCl2 and 0.3 units Taq polymerase apart for Tur4_87, Tur4_98, Tur4_128 and Tur4_142, where the concentrations were 0.125 mM dTNPs, 2.5 mM MgCl2 and 0.5 units Taq polymerase. Primer concentrations are indicated in the below table. Cycle conditions were as follow: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, annealing temperature for 30 s (see below table), and 72 °C for 45 s, followed by a final 72 °C extension for 7 min. Amplified products were screened on 6% polyacrylamide gels. Allele sizes were determined by eye using a size standard and alleles from reference samples. For the 7 loci analyzed on the 3730 XL ABI sequencer, amplification and electrophoresis were performed by Genoscreen (Lille, France) with conditions modified from Vollmer (2011). Each 25 µL PCR reaction contained 1 µl of extracted DNA, 1X reaction buffer, 0.24 mM dNTPs, 1.5 mM MgCl2 and 1 unit Taq polymerase. Primer concentrations are indicated in the below table. Cycle conditions were as follow: 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by a final 72 °C extension for 10 min. A LIZ500 size standard was used and allele sizes were scored by eye using Peakscanner (Applied Biosystems). A binning procedure as described in Rosel *et al.* (2009) was performed in order to ensure consistency of the scorings.

Table with the characteristics of each microsatellite locus. Loci screened on the LICOR 4300 DNA analyzer were only co-loaded for genotyping (Multiplex sequencer). Loci screened on a 3730XL ABI DNA sequencer were multiplexed for PCR (Multiplex PCR) and co-loaded for genotyping (Multiplex sequencer).

Markers	Reference	primers 5' - 3' (R and F)	Motif	allele size ranges	method	primer [C] (all or R/F/F* or R/F*) in µM	Annealing T° in °C	Multiplex PCR	Multiplex sequencer	Comments
EV37	Valsecchi & Amos 1996 - Vollmer 2011	AGCTTGATTTGGAAGTCATGA GTTTTAGTAGAGCCGTGATAAAGTGC	$(AC)_{24}$	196-250	ABI	0.24	55	c	2	Dye = 6FAM dilution 1/25
KMW12a	Hoelzel *et al*. 1998 - Bourret *et al*. 2008	CCATACAATCCAGCAGTC CACTGCAGAATGATGACC	$(CA)_n$	144-168	LICOR	0.125/ 0.075/ 0.05	46	-	8	
MK5	Krützen *et al.* 2001 - Vollmer 2011	CTCAGAGGGAAATGAGGCTG GTTTTGTCTAGAGGTCAAAGCCTTCC	$(TG)_{13}CT(TG)_2$ $CA(TG)_2(TA)_2$ $(TG)_4$	205-243	ABI	0.2	55	b	1	Dye = VIC dilution 1/20
MK6	Krützen *et al.* 2001 - Vollmer 2011	GTCCTCTTTCCAGGTGTAGCC GCCCACTAAGTATGTTGCAGC	$(GT)_{17}$	145-191	ABI	0.2	55	a	1	Dye = NED dilution 1/10
MK8	Krützen *et al.* 2001 - Vollmer 2011	TCCTGGAGCATCTTATAGTGGC GTTTCTCTTTGACATGCCCTCACC	$(CA)_{23}$	87-117	ABI	0.2	55	a	1	Dye = 6FAM dilution 1/10

MK9	Krützen *et al.* 2001 - Vollmer 2011	CATAACAAAGTGGGATGACTCC GTTTTTATCCTGTTGGCTGCAGTG	$(CA)_{17}$	166-182	ABI	0.4	55	a	1	Dye = 6FAM dilution 1/10
Tur4_87	Nater *et al.* 2009	CCCCATATGATGCCTTTGTAAGTCC AATTCCTTGTAACAAACCTCTTTATCT	$(GATA)_8$	182-202	LICOR	0.225/ 0.225	61	-	9	
Tur4_98	Nater *et al.* 2009	GTCCCCAGAACTTAGCACACTGTC CAACTGGGGTCCAAAGAAAGAAG	$(GATG)_{10}$	172-204	LICOR	0.225/ 0.225	63	-	10	
Tur4_128	Nater *et al.* 2009	ACGTGCGCATGTCTTTGTCTTAT CTTTGGACGGGGAGTAGAACCTA	$(GATA)_{11}$	280-304	LICOR	0.225/ 0.225	62	-	9	
Tur4_142	Nater *et al.* 2009	GGCCCCCTTTTCCATCCTCA CCAGCCCCCAAAATCACGAGT	$(GATA)_9$	320-340	LICOR	0.225/ 0.225	61	-	10	
TexVet5	Rooney *et al.* 1999 - Vollmer 2011	GATTGTGCAAATGGAGACA GTTTTTGAGATGACTCCTGTGGG	$(CA)_{24}$	201-223	ABI	0.125/ 0.075/ 0.05	55	c	2	Dye = VIC dilution 1/25
TexVet7	Rooney *et al.* 1999 - Vollmer 2011	TGCACTGTAGGGTGTTCAGCAG CTTAATTGGGGGCGATTTCAC	$(CA)_{12}$	162-178	ABI	0.2	55	b	1	Dye = PET dilution 1/20
Ttr04	Rosel *et al.* 2005	CTGACCAGGCACTTTCCAC GTTTGTTTCCCAGGATTTTAGTGC	$(CA)_{25}$	106-128	LICOR	0.125/ 0.075/ 0.05	60	-	4	
Ttr11	Rosel *et al.* 2005	CTTTCAACCTGGCCTTTCTG GTTTGGCCACTACAAGGGAGTGAA	$(CA)_{21}$	194-226	LICOR	0.125/ 0.075/ 0.05	62	-	8	
Ttr19	Rosel *et al.* 2005	TGGGTGGACCTCATCAAATC GTTTAAGGGCTGTAAGAGG	$(CA)_{17}$	174-202	LICOR	0.125/ 0.075/ 0.05	60	-	5	
Ttr34	Rosel *et al.* 2005	GCACATGAGTATGTGGACAGG GTTTCCTCCTTGGGAGTGTCCTCT	$(CA)_{19}$	182-204	LICOR	0.125/ 0.075/ 0.05	58	-	-	
Ttr48	Rosel *et al.* 2005	AAGAGGATGCAAATGGCAAG GTTTGGTAAGAAAATACCAAAGTCC	$(CA)_{18}$	132-144	LICOR	0.125/ 0.075/ 0.05	58	-	6	

Ttr58	Rosel *et al.* 2005	TGGGTCTTGAGGGGTCTG GTTTGCTGAGGCTCCTTGTTGG	$(CA)_{17}$	168-196	LICOR	0.125/ 0.075/ 0.05	60	-	4	
Ttr63	Rosel *et al.* 2005	CAGCTTACAGCCAAATGAGAG GTTTCTCCATGGCTGAGTCATCA	$(CA)_{34}$	86-140	LICOR	0.125/ 0.075/ 0.05	60	-	4	
TtrFF6	Rosel *et al.* 2005	AAGTAAGTGCTCCTTTGACTGG GTTTGGCAGAGAGATATTAGGACAGC	$(CA)_{20}$	134-174	LICOR	0.125/ 0.075/ 0.05	54	-	7	
Tut01	current study	CTGTTGTTGCCTCAATTTGC CCCATAGGACATATCCCACA	$(TG)_{11}$	117-125	LICOR	0.125/ 0.075/ 0.05	56	-	5	
Tut02	current study	CATTTGTTGGGAAGCTGTTG AGTGGGTTGACACATTCCCT	$(AC)_{11}$	181-209	LICOR	0.125/ 0.075/ 0.05	56	-	3	
Tut05	current study	GTATGCCTTGCTTTTGGTGC TGGGAGGTATGTCTGCAATAA	$(AC)_{13}$	154-166	LICOR	0.125/ 0.075/ 0.05	56	-	7	
Tut08	current study	AAGTTCCTAATTTCCCACCCA ACTTGTGTTTGCCTGCCTGT	$(AC)_{15}$	149-175	LICOR	0.125/ 0.075/ 0.05	56	-	3	
Tut09	current study	TAGGCTGGCAGAACACAAAG TGATTGTTTTCCTTCCTCGTG	$(AC)_{15}$	149-167	LICOR	0.125/ 0.075/ 0.05	56	-	6	

Notes: [C] = concentration and * indicated that the primer is marked.

Appendix A4.2. New microsatellite discovery method.

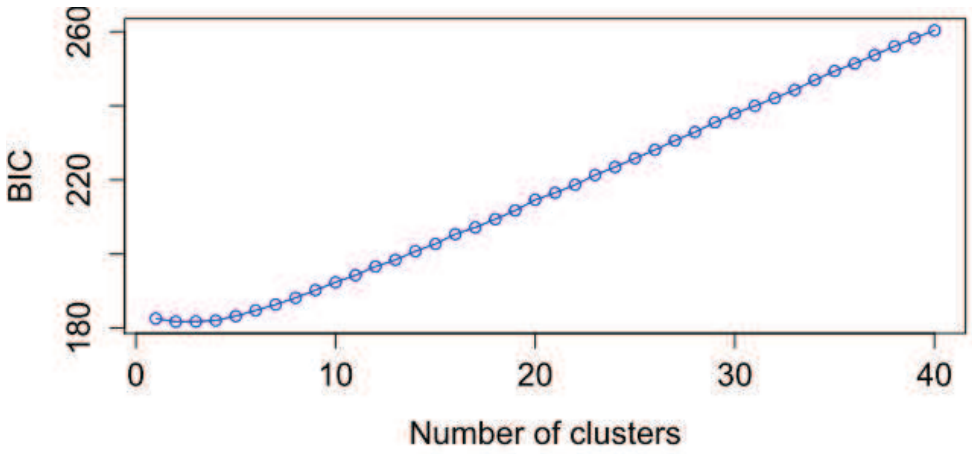
Total genomic DNA was isolated from 13 individuals randomly selected between Scotland and the Mediterranean Sea using NucleoSpin Tissue kits (Macherey-Nagel) following the manufacturer's protocol and sent to GenoScreen, France (www.genoscreen.com). A total of 1 µg was used for the development of microsatellite library through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries, as described in Malausa *et al.* (2011). Briefly, total DNA was enriched for AG, AC, AAC, AAG, AGG, ACG, ACAT, and ATCT repeat motifs and subsequently amplified. PCR products were purified, quantified, and GsFLX libraries were then constructed following manufacturer's protocols (Roche Diagnostics) and sequenced on a GsFLX-PTP. The bioinformatics program QDD (Meglécz *et al.* 2010) was used to filter for redundancy, resulting in a final set of sequences from which it was able to design primers. Finally, among 4660 sequences comprising a microsatellites motif, 194 primer sets were designed. We tested 13 primer sets on a LICOR 4300 sequencer and optimized the PCR and genotyping conditions for 6 primer sets (see Appendix A4.1).

Appendix A4.3. Microsatellite loci and their characteristics for bottlenose dolphins in the Normano-Breton gulf. The number of allele (NA) and allele richness (AR) were calculated in FSTAT 2.9.3. (Goudet 1995). Observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated in Arlequin (Michalakis & Excoffier 1996). F_{IS} and significance levels were estimated in Geneopop on the web version 4.2 (Raymond & Rousset 1995; Rousset 2008).

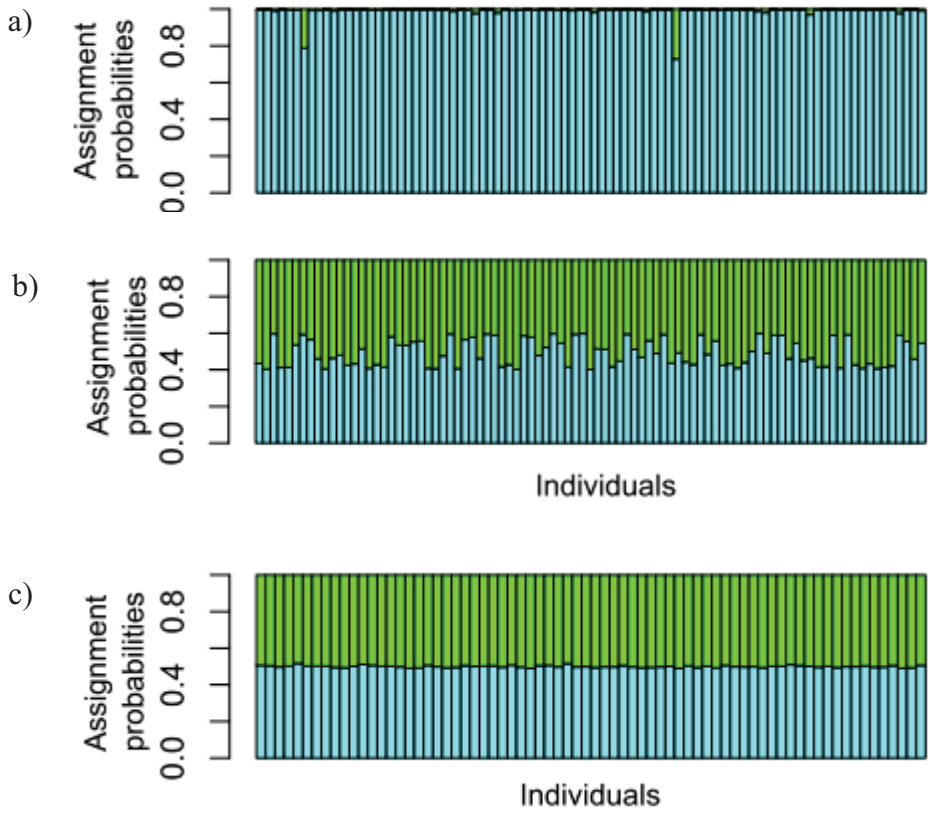
Locus	NA	AR	*Ho*	*He*	F_{IS} W&C	F_{IS} *P*-values
Tut08	8	7.977	0.85227	0.80481	-0.0593	0.5865
Tut02	8	8.000	0.78409	0.79481	0.0136	0.4371
Ttr34	6	6.000	0.82022	0.77649	-0.0567	0.7401
Ttr58	5	4.966	0.64045	0.64305	0.0041	0.6758
Ttr04	5	5.000	0.64773	0.66909	0.0321	0.4033
Ttr63	9	8.999	0.75281	0.73592	-0.0231	0.8455
Tut01	3	2.999	0.33708	0.35523	0.0514	0.7184
Ttr19	4	4.000	0.43182	0.39643	-0.0898	0.4822
Tut05	2	2.000	0.43820	0.41116	-0.0662	0.6098
TtrFF6	6	6.000	0.66292	0.71542	0.0738	0.7613
Tut09	5	4.999	0.34831	0.36990	0.0587	0.73
KMW12a	3	3.000	0.38202	0.43344	0.1192	0.4331
TA67	3	3.000	0.47727	0.52331	0.0884	0.436
TA74	4	4.000	0.60674	0.56948	-0.0658	0.9884
TA69	4	4.000	0.67416	0.66749	-0.01	0.5704
TA78	4	3.965	0.24719	0.27150	0.09	0.6135
Ttr11	6	5.999	0.51136	0.55117	0.0726	0.5676
Ttr48	7	6.966	0.70787	0.71726	0.0132	0.477
EV37	16	16.000	0.90698	0.89263	-0.0162	0.8053
MK5	6	6.000	0.78409	0.76435	-0.026	0.4153
MK6	6	6.000	0.76404	0.66305	-0.1533	0.3187
MK8	4	4.000	0.74157	0.74881	0.0097	0.9942
MK9	4	3.966	0.25843	0.24180	-0.0692	0.0685
TexVet5	5	5.000	0.63218	0.62461	-0.0122	0.6371
TexVet7	4	3.999	0.44944	0.44214	-0.0166	0.4095
Mean	5.480	5.467	0.59437	0.59133	-0.001504	0.5890
SD	2.786	3.292	0.19267	0.18181	0.0652	

Appendix A4.4. PCR conditions for the amplification of a portion (682-bp) of the mitochondrial control region.

Each 25 µL PCR reaction contained 5 µL of extracted DNA, 1X reaction Buffer, 0.25 mM dNTPs, 2mM MgCl2, 0.125 µM of each primer and 0.5 units Taq polymerase. Cycle conditions were as follows: 94 °C for 3 min followed by 39 cycles of 94 °C for 30 s, 51 °C for 30 s, 72 °C for 45 s, followed by a final 72 °C extension for 7 min. PCR products were sent to Genoscreen (Lille, France) for purification and sanger sequencing for both strands on an 3730XL ABI sequencer.

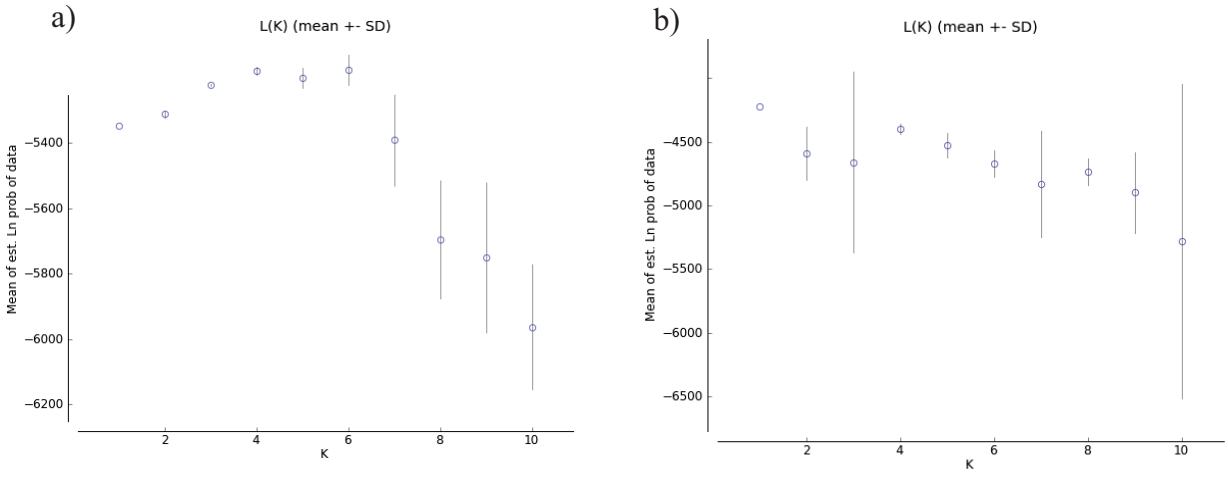


Appendix A4.5. Selection of the optimal number of clusters for the DAPC analysis using the BIC (Bayesian Information Criterion).

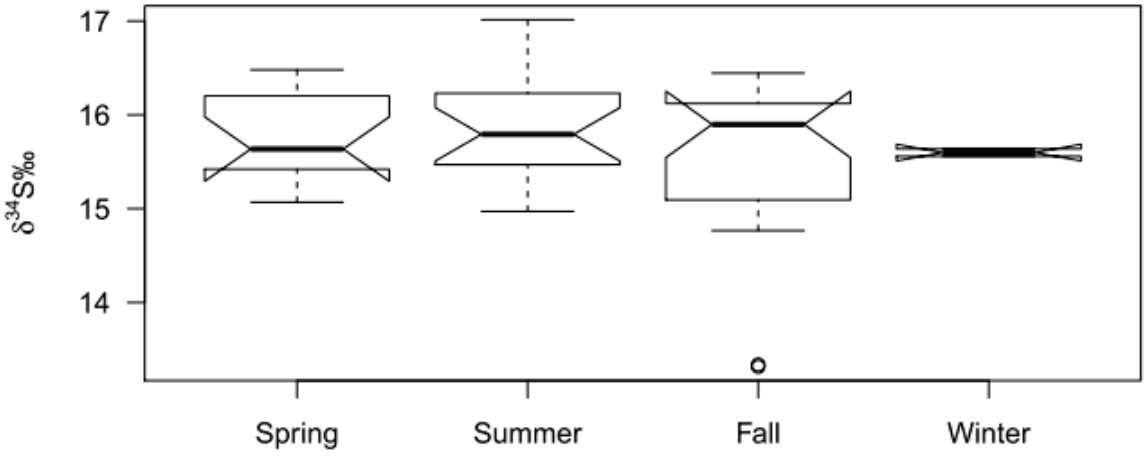


Appendix A4.6. Membership proportions of individual bottlenose dolphins inferred for *K* = 2 using a) TESS, b) STRUCTURE with all the dataset and c) STRUCTURE with the dataset where one individual per pair of closely related individuals was removed.

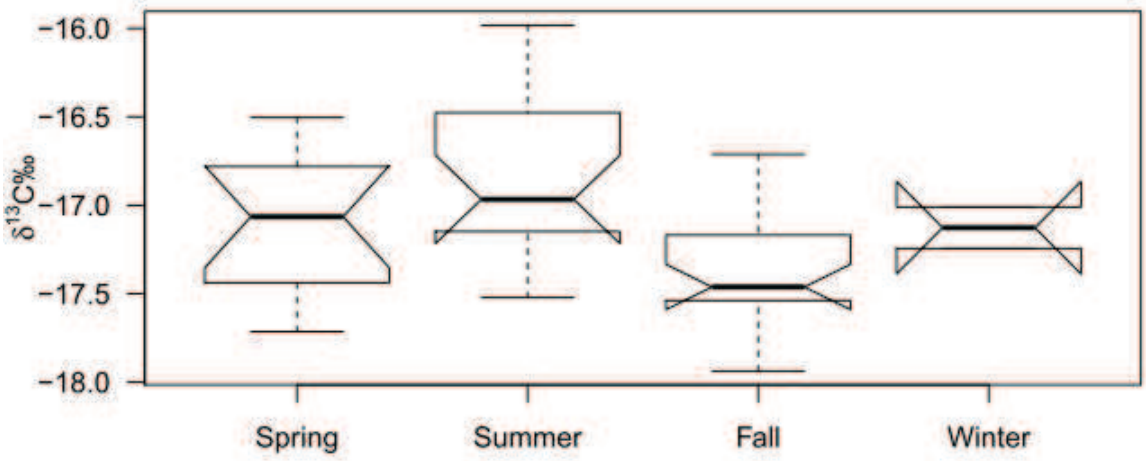
Each vertical column corresponds to one individual, with the colors representing the membership proportions to each of the two clusters. The three barplots indicate that *K* = 1.



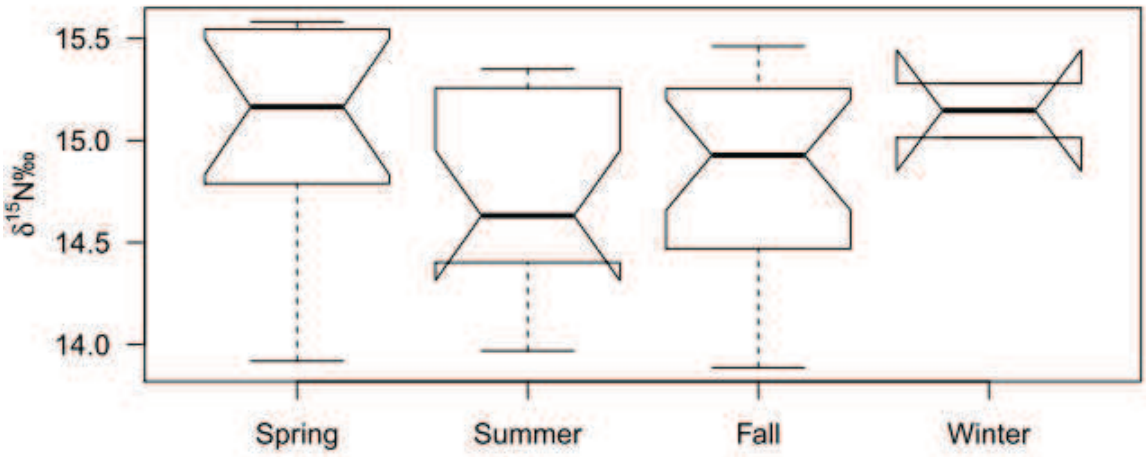
Appendix A4.7. STRUCTURE plots of the log probability of the data [*LnP*(D)] given values for *K* of 1 to 10 for the analyses with admixture and correlated allele frequencies for a) all the dataset and b) the dataset where one individual per pair of closely related individuals was removed.



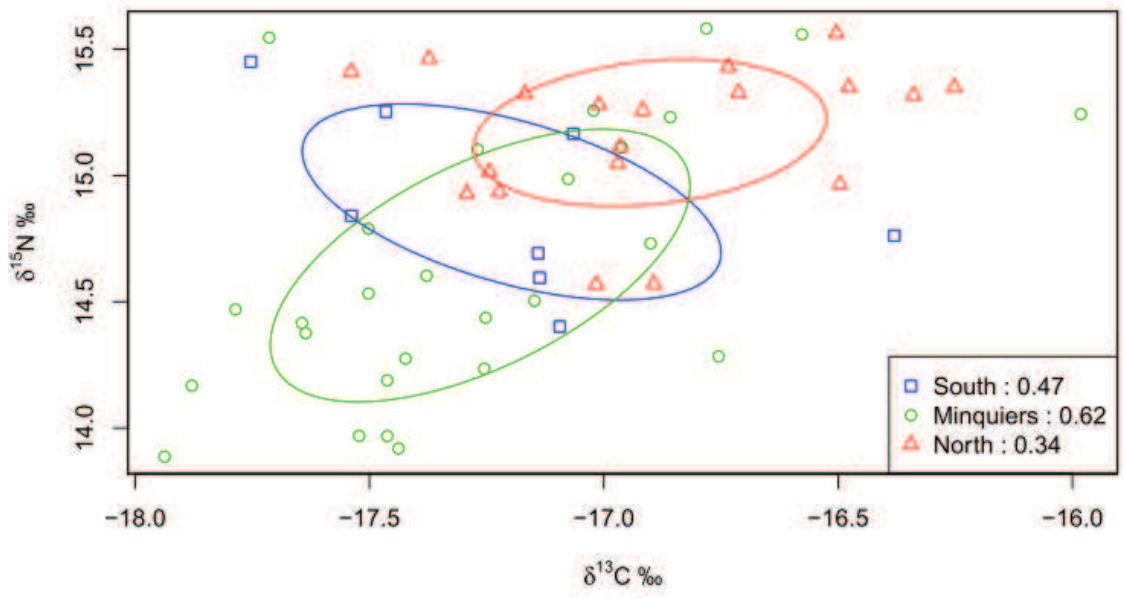
Appendix A4.8a. $\delta^{34}S$ signature (‰) variations according to season for individuals included in social structure analyses (N = 54).



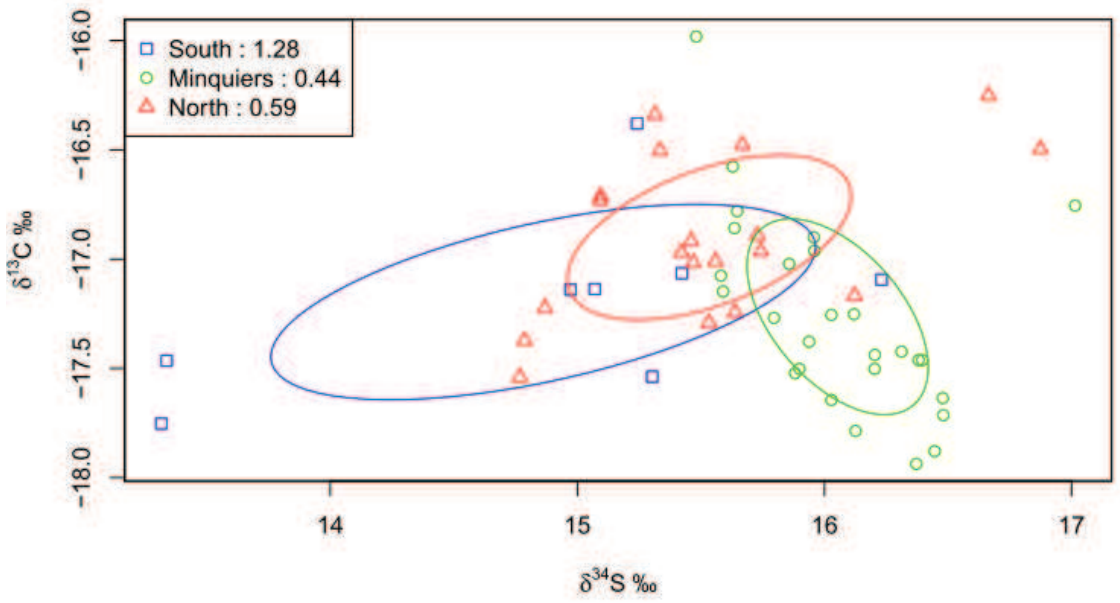
Appendix A4.8b. $\delta^{13}C$ signature (‰) variations according to season for individuals included in social structure analyses (N = 54).



Appendix A4.8c. $\delta^{15}N$ signature (‰) variations according to season for individuals included in social structure analyses (N = 54).

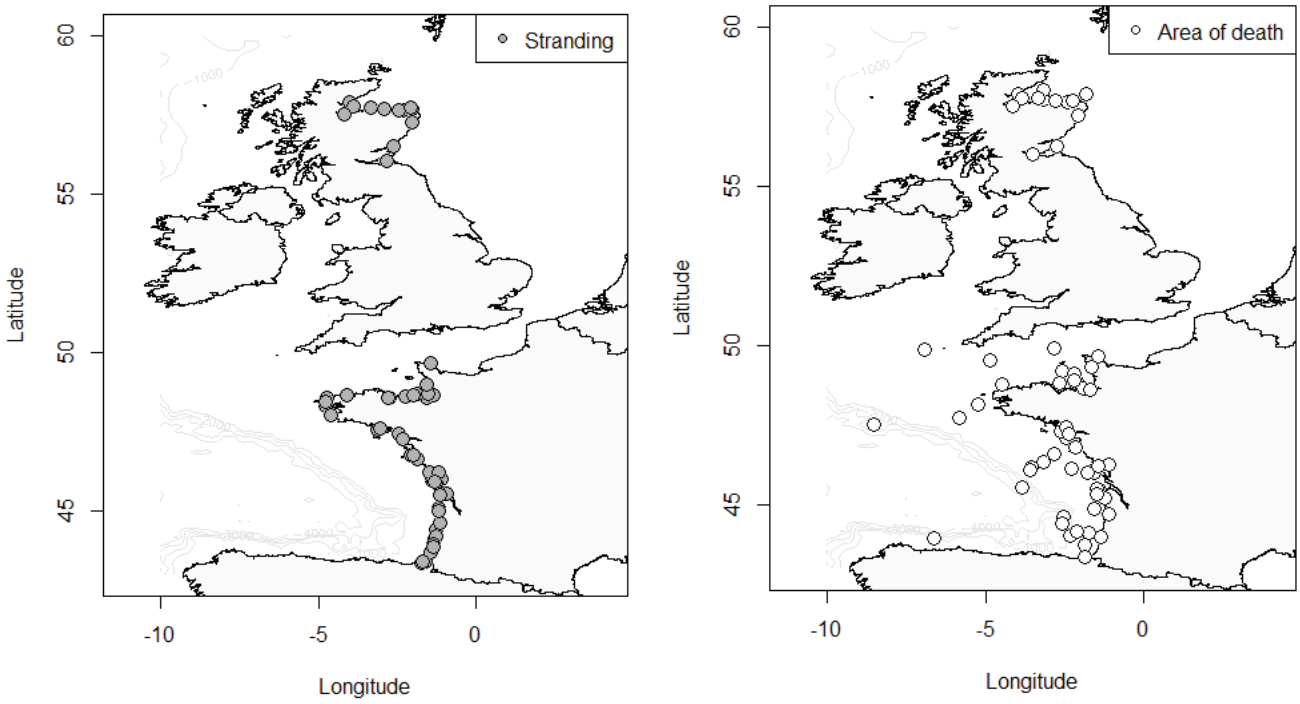


Appendix A4.9a. $\delta^{15}N$ and $\delta^{13}C$ signatures for each social group of bottlenose dolphins. Solid lines indicate Standard Ellipses Areas corrected for small sample sizes (SEA_c). Area values (‰²) are given in the legend.



Appendix A4.9b. $\delta^{13}C$ and $\delta^{34}S$ signatures for each social group of bottlenose dolphins. Solid lines indicate Standard Ellipses Areas corrected for small sample sizes (SEA_c). Area values (‰²) are given in the legend.

2) Appendix Chapter 5



Appendix A5.1. Map of stranding locations (left) for individuals for which we applied a drift prediction model and map of their most likely area of death (right).

Appendix A5.2. List of haplotypes obtained from GENBANK and used for the North Atlantic basin mtDNA haplotype network. It includes information on accession numbers (GENBANK), sampling locations (Origin with NWA = North-West Atlantic) and the articles where these sequences were reported and/or analyzed (Sellas *et al.* 2005; Quérouil *et al.* 2007; Kingston *et al.* 2009; Rosel *et al.* 2009; Vollmer 2011; Litz *et al.* 2012).

Name	Type	GENBANK	Origin	Article 1	Article 2	Article 3
OTtr10	haplotype	GQ504053	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
OTtr11	haplotype	GQ504074	NWA	Kingston *et al.* 2009	Vollmer 2011	
OTtr12	haplotype	GQ504054	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	Vollmer 2011
OTtr13	haplotype	GQ504075	NWA	Kingston *et al.* 2009		
OTtr14	haplotype	GQ504076	NWA	Kingston *et al.* 2009		
OTtr15	haplotype	GQ504077	NWA	Kingston *et al.* 2009	Vollmer 2011	
OTtr16	haplotype	GQ504078	NWA	Kingston *et al.* 2009		
OTtr17	haplotype	GQ504079	NWA	Kingston *et al.* 2009		
OTtr18	haplotype	GQ504080	NWA	Kingston *et al.* 2009		
OTtr19	haplotype	GQ504083	NWA	Kingston *et al.* 2009		
OTtr2	haplotype	GQ504065	NWA	Kingston *et al.* 2009	Vollmer 2011	
OTtr20	haplotype	GQ504084	NWA	Kingston *et al.* 2009		
OTtr21	haplotype	GQ504085	NWA	Kingston *et al.* 2009	Vollmer 2011	
OTtr22	haplotype	GQ504086	NWA	Kingston *et al.* 2009		
OTtr23	haplotype	GQ504087	NWA	Kingston *et al.* 2009	Vollmer 2011	
OTtr24	haplotype	GQ504088	NWA	Kingston *et al.* 2009	Vollmer 2011	
OTtr25	haplotype	GQ504092	NWA	Kingston *et al.* 2009		
OTtr26	haplotype	GQ504089	NWA	Kingston *et al.* 2009		
OTtr27	haplotype	GQ504091	NWA	Kingston *et al.* 2009		
OTtr28	haplotype	GQ504055	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	

OTtr29	haplotype	GQ504094	NWA	Kingston *et al.* 2009	
OTtr3	haplotype	GQ504067	NWA	Kingston *et al.* 2009	Vollmer 2011
OTtr30	haplotype	GQ504096	NWA	Kingston *et al.* 2009	Vollmer 2011
OTtr31	haplotype	GQ504097	NWA	Kingston *et al.* 2009	
OTtr32	haplotype	GQ504098	NWA	Kingston *et al.* 2009	Vollmer 2011
OTtr34	haplotype	GQ504066	NWA	Kingston *et al.* 2009	
OTtr35	haplotype	GQ504081	NWA	Kingston *et al.* 2009	
OTtr36	haplotype	GQ504082	NWA	Kingston *et al.* 2009	
OTtr37	haplotype	GQ504090	NWA	Kingston *et al.* 2009	Vollmer 2011
OTtr38	haplotype	GQ504093	NWA	Kingston *et al.* 2009	
OTtr39	haplotype	GQ504095	NWA	Kingston *et al.* 2009	
OTtr4	haplotype	GQ504068	NWA	Kingston *et al.* 2009	Vollmer 2011
OTtr40	haplotype	GQ504099	NWA	Kingston *et al.* 2009	
OTtr41	haplotype	GQ504106	NWA	Kingston *et al.* 2009	Vollmer 2011
OTtr42	haplotype	GQ504111	NWA	Kingston *et al.* 2009	
OTtr43	haplotype	GQ504112	NWA	Kingston *et al.* 2009	
OTtr44	haplotype	GQ504056	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009
OTtr45	haplotype	GQ504104	NWA	Kingston *et al.* 2009	
OTtr46	haplotype	GQ504105	NWA	Kingston *et al.* 2009	
OTtr47	haplotype	GQ504113	NWA	Kingston *et al.* 2009	
OTtr48	haplotype	GQ504057	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009
OTtr49	haplotype	HQ383685	Gulf of Mexico	Litz *et al.* 2012	
OTtr5	haplotype	GQ504069	NWA	Kingston *et al.* 2009	
OTtr6	haplotype	GQ504070	NWA	Kingston *et al.* 2009	
OTtr69	haplotype	HQ383684	NWA	Litz *et al.* 2012	
OTtr7	haplotype	GQ504071	NWA	Kingston *et al.* 2009	Vollmer 2011
OTtr8	haplotype	GQ504072	NWA	Kingston *et al.* 2009	
OTtr9	haplotype	GQ504073	NWA	Kingston *et al.* 2009	Vollmer 2011
Ttr09	haplotype	DQ845450	NWA	Kingston *et al.* 2009	

Ttr1	haplotype	GQ504040	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	Vollmer 2011
Ttr11	haplotype	GQ504046	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr12	haplotype	GQ504047	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr13	haplotype	GQ504048	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr15	haplotype	GQ504049	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	Vollmer 2011
Ttr16	haplotype	AY997309	GOM	Sellas *et al.* 2005	Vollmer 2011	
Ttr2	haplotype	AY997308	GOM	Sellas *et al.* 2005	Vollmer 2011	
Ttr28	haplotype	GQ504059	NWA	Kingston *et al.* 2009		
Ttr29	haplotype	GQ504052	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr3	haplotype	GQ504041	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr31	haplotype	GQ504100	NWA	Kingston *et al.* 2009		
Ttr32	haplotype	GQ504101	NWA	Kingston *et al.* 2009	Litz *et al.* 2012	
Ttr37	haplotype	GQ504109	NWA	Kingston *et al.* 2009		
Ttr38	haplotype	GQ504110	NWA	Kingston *et al.* 2009		
Ttr39	haplotype	GQ504102	NWA	Kingston *et al.* 2009		
Ttr4	haplotype	GQ504042	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	Vollmer 2011
Ttr40	haplotype	GQ504103	NWA	Kingston *et al.* 2009	Litz *et al.* 2012	
Ttr41	haplotype	HQ383686	Gulf of Mexico	Litz *et al.* 2012		
Ttr5	haplotype	GQ504043	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr6	haplotype	GQ504044	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr7	haplotype	GQ504045	NWA	Kingston *et al.* 2009	Rosel *et al.* 2009	
Ttr8	haplotype	GQ504058	NWA	Kingston et al. 2009		
TT087	voucher	DQ525362	Azores	Quérouil *et al.* 2007		
TT086	voucher	DQ525361	Azores	Quérouil *et al.* 2007		
TT085	voucher	DQ525360	Azores	Quérouil *et al.* 2007		
TT084	voucher	DQ525359	Azores	Quérouil *et al.* 2007		
TT083	voucher	DQ525358	Azores	Quérouil *et al.* 2007		
TT071	voucher	DQ525357	Azores	Quérouil *et al.* 2007		
TT080	voucher	DQ073717	Azores	Quérouil *et al.* 2007		

TT079	voucher	DQ073716	Azores	Quérouil *et al.* 2007
TT078	voucher	DQ073715	Azores	Quérouil *et al.* 2007
TT077	voucher	DQ073714	Azores	Quérouil *et al.* 2007
TT076	voucher	DQ073713	Azores	Quérouil *et al.* 2007
TT075	voucher	DQ073712	Azores	Quérouil *et al.* 2007
TT074	voucher	DQ073711	Azores	Quérouil *et al.* 2007
TT073	voucher	DQ073710	Azores	Quérouil *et al.* 2007
TT072	voucher	DQ073709	Azores	Quérouil *et al.* 2007
TT070	voucher	DQ073708	Azores	Quérouil *et al.* 2007
TT069	voucher	DQ073707	Azores	Quérouil *et al.* 2007
TT067	voucher	DQ073706	Azores	Quérouil *et al.* 2007
TT066	voucher	DQ073705	Azores	Quérouil *et al.* 2007
TT065	voucher	DQ073704	Azores	Quérouil *et al.* 2007
TT064	voucher	DQ073703	Azores	Quérouil *et al.* 2007
TT063	voucher	DQ073702	Azores	Quérouil *et al.* 2007
TT061	voucher	DQ073701	Azores	Quérouil *et al.* 2007
TT060	voucher	DQ073700	Azores	Quérouil *et al.* 2007
TT059	voucher	DQ073699	Azores	Quérouil *et al.* 2007
TT058	voucher	DQ073698	Azores	Quérouil *et al.* 2007
TT057	voucher	DQ073697	Azores	Quérouil *et al.* 2007
TT056	voucher	DQ073696	Azores	Quérouil *et al.* 2007
TT055	voucher	DQ073695	Azores	Quérouil *et al.* 2007
TT054	voucher	DQ073694	Azores	Quérouil *et al.* 2007
TT053	voucher	DQ073693	Azores	Quérouil *et al.* 2007
TT052	voucher	DQ073692	Azores	Quérouil *et al.* 2007
TT051	voucher	DQ073691	Azores	Quérouil *et al.* 2007
TT050	voucher	DQ073690	Azores	Quérouil *et al.* 2007
TT049	voucher	DQ073689	Azores	Quérouil *et al.* 2007
TT048	voucher	DQ073688	Azores	Quérouil *et al.* 2007

TT047	voucher	DQ073687	Azores	Quérouil *et al.* 2007
TT046	voucher	DQ073686	Azores	Quérouil *et al.* 2007
TT045	voucher	DQ073685	Azores	Quérouil *et al.* 2007
TT044	voucher	DQ073684	Azores	Quérouil *et al.* 2007
TT043	voucher	DQ073683	Azores	Quérouil *et al.* 2007
TT042	voucher	DQ073682	Azores	Quérouil *et al.* 2007
TT041	voucher	DQ073681	Azores	Quérouil *et al.* 2007
TT040	voucher	DQ073680	Azores	Quérouil *et al.* 2007
TT039	voucher	DQ073679	Azores	Quérouil *et al.* 2007
TT038	voucher	DQ073678	Azores	Quérouil *et al.* 2007
TT037	voucher	DQ073677	Azores	Quérouil *et al.* 2007
TT036	voucher	DQ073676	Azores	Quérouil *et al.* 2007
TT035	voucher	DQ073675	Azores	Quérouil *et al.* 2007
TT034	voucher	DQ073674	Azores	Quérouil *et al.* 2007
TT033	voucher	DQ073673	Azores	Quérouil *et al.* 2007
TT032	voucher	DQ073672	Azores	Quérouil *et al.* 2007
TT031	voucher	DQ073671	Azores	Quérouil *et al.* 2007
TT030	voucher	DQ073670	Azores	Quérouil *et al.* 2007
TT029	voucher	DQ073669	Azores	Quérouil *et al.* 2007
TT028	voucher	DQ073668	Azores	Quérouil *et al.* 2007
TT027	voucher	DQ073667	Azores	Quérouil *et al.* 2007
TT026	voucher	DQ073666	Azores	Quérouil *et al.* 2007
TT025	voucher	DQ073665	Azores	Quérouil *et al.* 2007
TT024	voucher	DQ073664	Azores	Quérouil *et al.* 2007
TT023	voucher	DQ073663	Azores	Quérouil *et al.* 2007
TT022	voucher	DQ073662	Azores	Quérouil *et al.* 2007
TT021	voucher	DQ073661	Azores	Quérouil *et al.* 2007
TT020	voucher	DQ073660	Azores	Quérouil *et al.* 2007
TT019	voucher	DQ073659	Azores	Quérouil *et al.* 2007

TT018	voucher	DQ073658	Azores	Quérouil *et al.* 2007
TT017	voucher	DQ073657	Azores	Quérouil *et al.* 2007
TT016	voucher	DQ073656	Azores	Quérouil *et al.* 2007
TT015	voucher	DQ073655	Azores	Quérouil *et al.* 2007
TT014	voucher	DQ073654	Azores	Quérouil *et al.* 2007
TT013	voucher	DQ073653	Azores	Quérouil *et al.* 2007
TT012	voucher	DQ073652	Azores	Quérouil *et al.* 2007
TT011	voucher	DQ073651	Azores	Quérouil *et al.* 2007
TT010	voucher	DQ073650	Azores	Quérouil *et al.* 2007
TT009	voucher	DQ073649	Azores	Quérouil *et al.* 2007
TT008	voucher	DQ073648	Azores	Quérouil *et al.* 2007
TT007	voucher	DQ073647	Azores	Quérouil *et al.* 2007
TT006	voucher	DQ073646	Azores	Quérouil *et al.* 2007
TT005	voucher	DQ073645	Azores	Quérouil *et al.* 2007
TT004	voucher	DQ073644	Azores	Quérouil *et al.* 2007
TT003	voucher	DQ073643	Azores	Quérouil *et al.* 2007
TT002	voucher	DQ073642	Azores	Quérouil *et al.* 2007
TT001	voucher	DQ073641	Azores	Quérouil *et al.* 2007
TT0106	voucher	FJ768019	Azores	Quérouil *et al.* 2007
TT0105	voucher	FJ768018	Azores	Quérouil *et al.* 2007
TT0104	voucher	FJ768017	Azores	Quérouil *et al.* 2007
TT0103	voucher	FJ768016	Azores	Quérouil *et al.* 2007
TT0102	voucher	FJ768015	Azores	Quérouil *et al.* 2007
TT0101	voucher	FJ768014	Azores	Quérouil *et al.* 2007
TT0100	voucher	FJ768013	Azores	Quérouil *et al.* 2007
TT099	voucher	FJ768012	Azores	Quérouil *et al.* 2007
TT098	voucher	FJ768011	Azores	Quérouil *et al.* 2007
TT097	voucher	FJ768010	Azores	Quérouil *et al.* 2007
TT096	voucher	FJ768009	Azores	Quérouil *et al.* 2007

TT095	voucher	FJ768008	Azores	Quérouil *et al.* 2007
TT094	voucher	FJ768007	Azores	Quérouil *et al.* 2007
TT093	voucher	FJ768006	Azores	Quérouil *et al.* 2007
TT092	voucher	FJ768005	Azores	Quérouil *et al.* 2007
TT091	voucher	FJ768004	Azores	Quérouil *et al.* 2007
TT090	voucher	FJ768003	Azores	Quérouil *et al.* 2007
TT089	voucher	FJ768002	Azores	Quérouil *et al.* 2007
TT088	voucher	FJ768001	Azores	Quérouil *et al.* 2007
TTM030	voucher	DQ525388	Madeira	Quérouil *et al.* 2007
TTM029	voucher	DQ525387	Madeira	Quérouil *et al.* 2007
TTM028	voucher	DQ525386	Madeira	Quérouil *et al.* 2007
TTM027	voucher	DQ525385	Madeira	Quérouil *et al.* 2007
TTM025	voucher	DQ525383	Madeira	Quérouil *et al.* 2007
TTM026	voucher	DQ525384	Madeira	Quérouil *et al.* 2007
TTM023	voucher	DQ525382	Madeira	Quérouil *et al.* 2007
TTM022	voucher	DQ525381	Madeira	Quérouil *et al.* 2007
TTM019	voucher	DQ525379	Madeira	Quérouil *et al.* 2007
TTM016	voucher	DQ525378	Madeira	Quérouil *et al.* 2007
TTM015	voucher	DQ525377	Madeira	Quérouil *et al.* 2007
TTM014	voucher	DQ525376	Madeira	Quérouil *et al.* 2007
TTM013	voucher	DQ525375	Madeira	Quérouil *et al.* 2007
TTM012	voucher	DQ525374	Madeira	Quérouil *et al.* 2007
TTM010	voucher	DQ525373	Madeira	Quérouil *et al.* 2007
TTM009	voucher	DQ525372	Madeira	Quérouil *et al.* 2007
TTM008	voucher	DQ525371	Madeira	Quérouil *et al.* 2007
TTM007	voucher	DQ525370	Madeira	Quérouil *et al.* 2007
TTM006	voucher	DQ525369	Madeira	Quérouil *et al.* 2007
TTM005	voucher	DQ525368	Madeira	Quérouil *et al.* 2007
TTM004	voucher	DQ525367	Madeira	Quérouil *et al.* 2007

TTM003	voucher	DQ525366	Madeira	Quérouil *et al.* 2007
TTM002	voucher	DQ525365	Madeira	Quérouil *et al.* 2007
TTM001	voucher	DQ525364	Madeira	Quérouil *et al.* 2007

Appendix A5.3. BayesAss settings.

As recommended by Rannala (2013) preliminary runs were first performed to adjust the Markov Chain Monte Carlo (MCMC) mixing parameters of migrations rates, allele frequencies and inbreeding coefficients to ensure proposal acceptance rates around 30%. We then performed 10 runs with a burnin of 1×10^6 iterations followed by 2×10^7 MCMC iterations and a sampling frequency of 1000. Trace files were plotted using Tracer (Rambaut & Drummond 2007) to check for convergence and mixing. Consistency of the results between the runs was also checked.

Appendix A5.4. Test for Hardy-Weinberg Equilibrium (HWE) deviation of each locus in each population and in the whole data set (*P*-values that are significant after sequential Bonferroni correction are highlighted in boldface). Inbreeding coefficient (F_{IS} W&C), Observed Heterozygosity (*Ho*), Expected Heterozygosity (*He*), Number of alleles (NA), Allele Richness (AR) and number of private alleles (PA) were also calculated for each locus in each population, and in the whole dataset when appropriate.

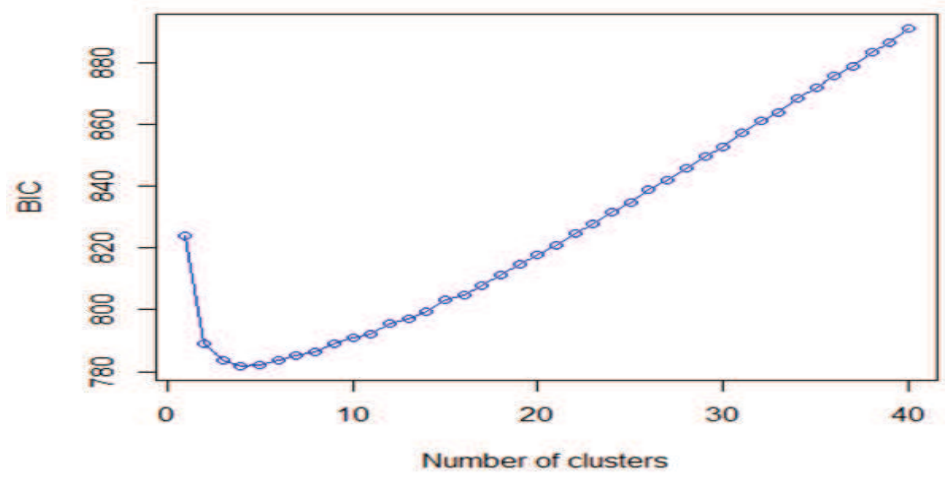
Population	Locus	HWE	F_{IS} W&C	*Ho*	*He*	NA	AR	PA
Coastal_South	Tut08	0.1985	0.0199	0.79130	0.80733	7	6.887	
Coastal_South	Tut02	0.1186	0.0381	0.76724	0.79747	9	8.446	
Coastal_South	Ttr34	0.5937	-0.0107	0.76724	0.75918	6	5.996	
Coastal_South	Ttr58	0.3785	0.0110	0.64407	0.65117	7	5.770	1
Coastal_South	Ttr04	0.7307	0.0386	0.66667	0.69333	7	6.050	
Coastal_South	Ttr63	0.0962	0.0055	0.74576	0.74984	10	9.714	
Coastal_South	Tut01	0.5553	0.0565	0.30252	0.32057	3	2.875	
Coastal_South	Ttr19	0.3767	0.0153	0.41379	0.42021	5	4.733	
Coastal_South	Tut05	0.6541	-0.0474	0.42373	0.40462	2	2.000	
Coastal_South	TtrFF6	0.4616	0.0690	0.64407	0.69163	6	5.837	
Coastal_South	Tut09	0.4878	0.1129	0.40678	0.45831	5	4.860	
Coastal_South	KMW12a	0.2246	0.1614	0.35294	0.42056	5	3.805	
Coastal_South	TA67	0.4042	0.1017	0.46154	0.51359	3	2.881	
Coastal_South	TA74	0.2993	0.0080	0.55932	0.56383	5	4.792	
Coastal_South	TA69	0.6265	-0.0212	0.64706	0.63366	4	4.000	
Coastal_South	TA78	0.0866	0.0931	0.29412	0.32419	4	3.643	
Coastal_South	Ttr11	0.6388	0.0730	0.54783	0.59077	7	5.862	
Coastal_South	Ttr48	0.2790	-0.0024	0.72034	0.71864	7	6.828	
Coastal_South	EV37	0.8947	-0.0112	0.91071	0.90070	16	15.106	1
Coastal_South	MK5	0.4758	-0.0065	0.75862	0.75377	8	6.722	

Coastal_South	MK6	0.2205	-0.0701	0.67521	0.63119	8	6.728	
Coastal_South	MK8	0.6726	0.0363	0.73729	0.76495	8	6.013	
Coastal_South	**MK9**	**0.0000**	**0.0767**	0.26050	0.28206	6	4.668	
Coastal_South	TexVet5	0.4072	0.0345	0.56522	0.58530	5	4.978	
Coastal_South	TexVet7	0.0746	-0.0271	0.48305	0.47036	5	4.778	
Coastal_North	Tut08	0.0135	0.1201	0.60274	0.68446	8	7.645	
Coastal_North	Tut02	0.0949	0.1228	0.64865	0.73883	6	5.756	
Coastal_North	Ttr34	0.8771	-0.0385	0.71622	0.68983	7	6.293	1
Coastal_North	Ttr58	0.6366	-0.0025	0.51351	0.51223	5	4.644	
Coastal_North	Ttr04	0.8408	0.0824	0.63636	0.69315	5	4.999	
Coastal_North	Ttr63	0.1289	0.0774	0.76389	0.82751	10	9.298	1
Coastal_North	Tut01	0.3209	0.1418	0.11842	0.13785	4	3.583	
Coastal_North	Ttr19	0.0071	0.2766	0.36111	0.49825	5	4.333	
Coastal_North	Tut05	0.4425	-0.0391	0.37333	0.35937	3	3.000	
Coastal_North	TtrFF6	0.5006	0.1693	0.47143	0.56680	5	4.588	
Coastal_North	Tut09	0.3530	0.0785	0.50649	0.54936	7	6.380	
Coastal_North	KMW12a	0.0592	0.0137	0.67105	0.68029	5	4.982	
Coastal_North	TA67	0.0268	0.0757	0.25974	0.28088	3	2.981	
Coastal_North	TA74	0.0169	0.2135	0.31507	0.40000	5	4.656	
Coastal_North	TA69	0.1650	0.0744	0.65789	0.71044	4	4.000	
Coastal_North	TA78	0.2048	0.1209	0.12987	0.14761	3	2.992	
Coastal_North	Ttr11	0.1286	0.0548	0.52632	0.55664	6	5.497	
Coastal_North	Ttr48	0.2248	0.1127	0.40000	0.45047	5	4.278	
Coastal_North	**EV37**	**0.0000**	**0.2113**	0.67606	0.85586	14	13.008	
Coastal_North	MK5	0.0578	0.1879	0.51351	0.63155	6	4.946	
Coastal_North	MK6	0.6809	0.0280	0.59722	0.61432	5	4.988	
Coastal_North	MK8	0.2736	0.1347	0.56000	0.64662	8	7.136	
Coastal_North	MK9	0.4551	0.0753	0.33766	0.36499	5	4.616	
Coastal_North	TexVet5	0.9644	0.0262	0.55405	0.56885	6	5.256	

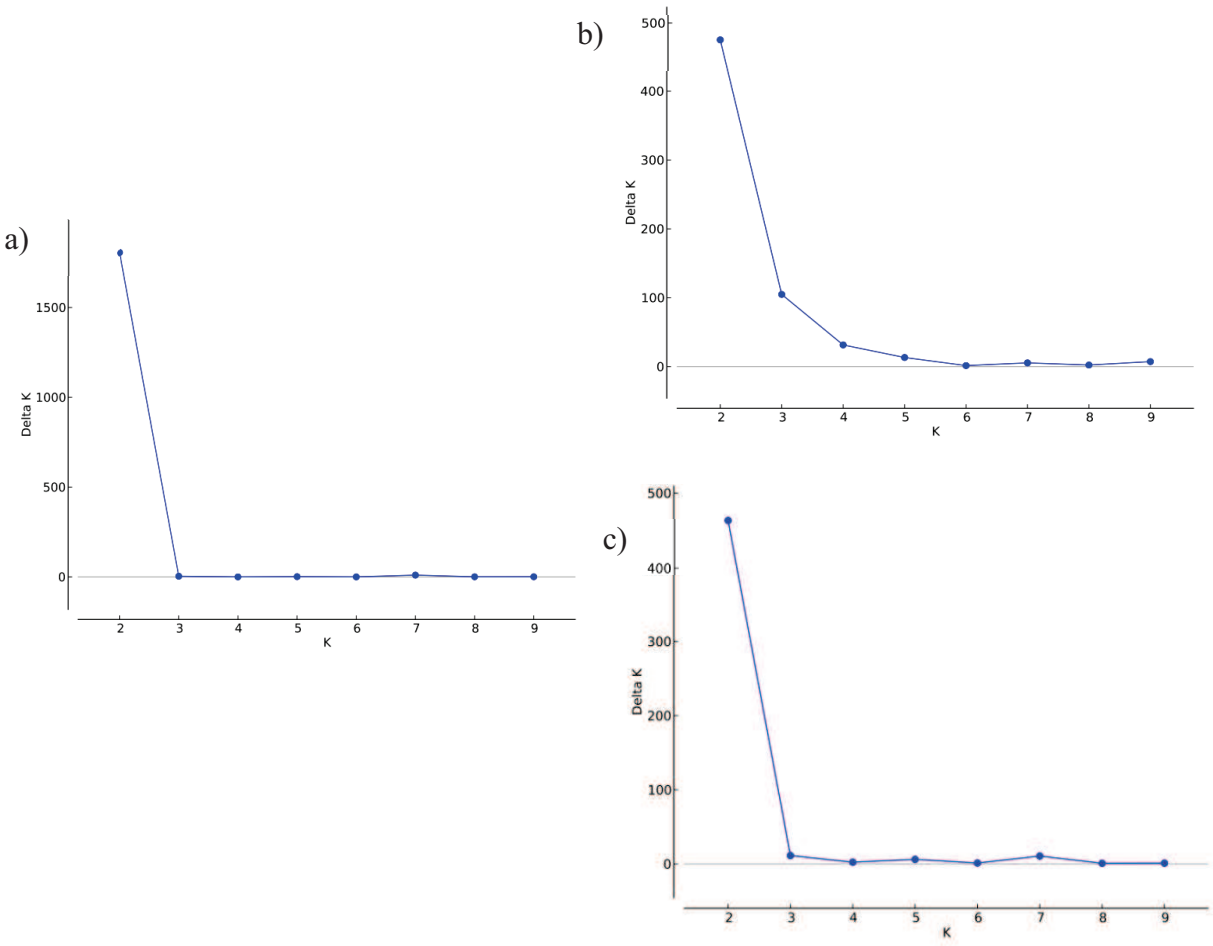
Coastal_North	TexVet7	0.0293	0.2990	0.24675	0.35133	4	3.808	
Pelagic_Atlantic	Tut08	0.5086	0.0297	0.83178	0.85714	10	9.678	1
Pelagic_Atlantic	Tut02	0.6151	0.0060	0.84906	0.85415	11	10.598	1
Pelagic_Atlantic	Ttr34	0.8011	0.0003	0.73585	0.73607	8	7.377	1
Pelagic_Atlantic	Ttr58	0.1191	0.0583	0.81132	0.86131	9	8.694	2
Pelagic_Atlantic	Ttr04	0.4610	-0.0057	0.83178	0.82708	11	10.024	2
Pelagic_Atlantic	Ttr63	0.2552	0.0678	0.78095	0.83750	18	15.074	5
Pelagic_Atlantic	Tut01	0.5801	0.0629	0.29245	0.31436	4	3.688	
Pelagic_Atlantic	Ttr19	0.0695	0.0099	0.79048	0.79836	9	8.950	1
Pelagic_Atlantic	Tut05	0.6862	0.0239	0.69811	0.71510	6	5.834	1
Pelagic_Atlantic	TtrFF6	0.4987	0.0366	0.79048	0.82037	14	11.913	7
Pelagic_Atlantic	Tut09	0.0087	0.1388	0.70476	0.81782	10	9.285	2
Pelagic_Atlantic	KMW12a	0.6432	-0.0010	0.76415	0.76339	9	7.856	2
Pelagic_Atlantic	TA67	0.8947	0.0194	0.50943	0.51945	4	3.453	1
Pelagic_Atlantic	TA74	0.8700	-0.0378	0.72642	0.70008	6	5.428	1
Pelagic_Atlantic	TA69	0.9777	0.0145	0.57547	0.58388	5	4.453	1
Pelagic_Atlantic	TA78	0.6608	0.0191	0.70755	0.72123	6	5.838	2
Pelagic_Atlantic	Ttr11	0.8135	0.0210	0.81731	0.83473	12	11.011	2
Pelagic_Atlantic	Ttr48	0.7863	-0.0119	0.82857	0.81891	7	6.996	
Pelagic_Atlantic	EV37	0.2226	0.0173	0.88571	0.90125	19	16.137	3
Pelagic_Atlantic	MK5	0.4678	0.0042	0.87500	0.87872	15	14.004	3
Pelagic_Atlantic	MK6	0.8890	0.0107	0.87619	0.88558	14	13.050	3
Pelagic_Atlantic	MK8	0.9393	-0.0195	0.79439	0.77930	10	9.143	
Pelagic_Atlantic	MK9	0.0520	0.0560	0.75701	0.80172	9	8.130	2
Pelagic_Atlantic	TexVet5	0.9919	-0.0132	0.86667	0.85546	11	9.860	2
Pelagic_Atlantic	TexVet7	0.8286	0.0165	0.74286	0.75530	9	7.744	3
Pelagic_Mediterranean	Tut08	0.7168	0.0626	0.78431	0.83615	7	7.000	
Pelagic_Mediterranean	Tut02	0.3378	0.0888	0.78431	0.86003	11	10.941	1
Pelagic_Mediterranean	Ttr34	0.0076	0.0227	0.67308	0.68857	7	6.918	1

Pelagic_Mediterranean	Ttr58	0.5696	0.0862	0.71154	0.77801	6	6.000	
Pelagic_Mediterranean	Ttr04	0.2713	0.0558	0.78846	0.83458	9	9.000	
Pelagic_Mediterranean	Ttr63	0.8224	-0.0470	0.88235	0.84314	15	14.761	1
Pelagic_Mediterranean	Tut01	0.0096	0.4013	0.15385	0.25597	3	3.000	
Pelagic_Mediterranean	Ttr19	0.7633	-0.0098	0.80000	0.79232	7	7.000	
Pelagic_Mediterranean	Tut05	0.7417	-0.0006	0.65385	0.65347	5	4.923	
Pelagic_Mediterranean	TtrFF6	0.2140	-0.1823	0.94118	0.79752	7	7.000	
Pelagic_Mediterranean	Tut09	0.1373	0.0753	0.66667	0.72044	7	6.997	
Pelagic_Mediterranean	KMW12a	0.5745	0.0246	0.63462	0.65049	7	6.918	
Pelagic_Mediterranean	TA67	0.9665	-0.0473	0.68627	0.65560	3	3.000	
Pelagic_Mediterranean	TA74	0.1093	0.0642	0.48077	0.51344	5	5.000	
Pelagic_Mediterranean	TA69	0.1125	0.0536	0.58824	0.62124	6	5.882	2
Pelagic_Mediterranean	TA78	0.1520	-0.0526	0.61538	0.58495	4	4.000	
Pelagic_Mediterranean	Ttr11	0.2417	0.0652	0.80392	0.85944	10	9.994	
Pelagic_Mediterranean	Ttr48	0.6703	0.0668	0.76471	0.81887	7	6.941	
Pelagic_Mediterranean	EV37	0.1685	0.0824	0.81250	0.88465	14	14.000	
Pelagic_Mediterranean	MK5	0.6142	-0.1452	0.84000	0.73455	11	10.958	
Pelagic_Mediterranean	MK6	0.2712	0.0787	0.82000	0.88929	16	15.916	3
Pelagic_Mediterranean	MK8	0.2731	0.0051	0.76471	0.76859	9	8.824	
Pelagic_Mediterranean	MK9	0.2229	0.1587	0.58824	0.69812	7	6.882	
Pelagic_Mediterranean	TexVet5	0.1395	0.0886	0.70588	0.77383	8	7.935	
Pelagic_Mediterranean	TexVet7	0.1042	0.1489	0.54902	0.64415	5	5.000	
ALL	**Tut08**	**0.0000**	**0.1006**	0.76286	0.84804	11	10.160	
ALL	**Tut02**	**0.0000**	**0.0957**	0.76923	0.85051	12	10.231	
ALL	Ttr34	0.4176	0.0306	0.73580	0.75897	10	7.217	
ALL	**Ttr58**	**0.0000**	**0.1217**	0.67797	0.77181	10	8.071	
ALL	**Ttr04**	**0.0002**	**0.0840**	0.73109	0.79806	11	8.830	
ALL	**Ttr63**	**0.0000**	**0.1035**	0.78000	0.86991	23	16.117	
ALL	Tut01	0.0628	0.1255	0.24370	0.27862	5	4.024	

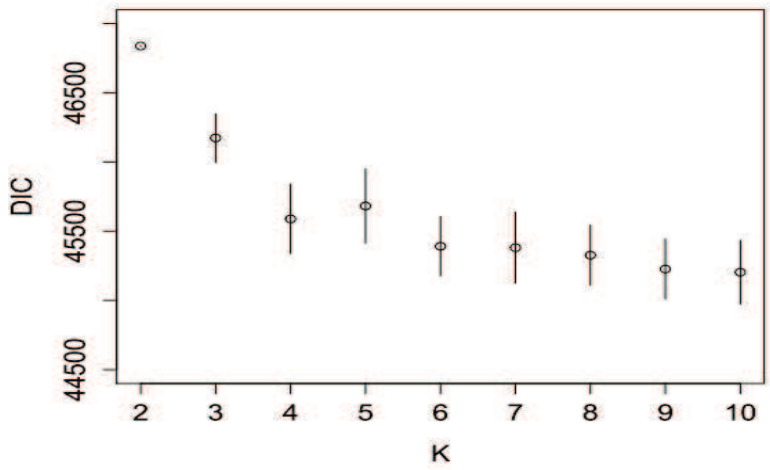
ALL	**Ttr19**	**0.0000**	**0.1537**	0.57349	0.67747	9	8.469
ALL	**Tut05**	**0.0000**	**0.2100**	0.52958	0.67017	6	5.332
ALL	**TtrFF6**	**0.0000**	**0.1046**	0.69828	0.77972	15	10.038
ALL	**Tut09**	**0.0000**	**0.2122**	0.55493	0.70417	10	8.932
ALL	**KMW12a**	**0.0000**	**0.0962**	0.59104	0.65384	9	6.737
ALL	**TA67**	**0.0002**	**0.1803**	0.46479	0.56686	4	3.137
ALL	TA74	0.2362	0.0549	0.54674	0.57846	6	5.080
ALL	TA69	0.0617	0.0339	0.61798	0.63962	7	4.409
ALL	**TA78**	**0.0001**	**0.1540**	0.43575	0.51496	6	5.227
ALL	**Ttr11**	**0.0000**	**0.1723**	0.66286	0.80065	12	9.936
ALL	**Ttr48**	**0.0000**	**0.1448**	0.68555	0.80143	7	6.993
ALL	**EV37**	**0.0000**	**0.0996**	0.84118	0.93404	25	20.013
ALL	**MK5**	**0.0014**	**0.0795**	0.75575	0.82095	15	11.883
ALL	**MK6**	**0.0000**	**0.1004**	0.74138	0.82405	19	13.774
ALL	**MK8**	**0.0000**	**0.0975**	0.72394	0.80206	10	9.023
ALL	**MK9**	**0.0000**	**0.1984**	0.46927	0.58526	9	7.388
ALL	**TexVet5**	**0.0000**	**0.0934**	0.67335	0.74266	11	9.520
ALL	**TexVet7**	**0.0006**	**0.1484**	0.51268	0.60189	9	6.384



Appendix A5.5. Selection of the optimal number of clusters for the DAPC analysis using the lowest BIC (Bayesian Information Criterion).



Appendix A5.6. Evanno plots of the STRUCTURE analyses separating (a) the coastal and pelagic groups, (b) the two coastal populations and (c) the two pelagic populations.



Appendix A5.7. Mean Deviance Information Criterion (and SD) values using the 10 replicate TESS runs for each *K* from 2 to 10.

Appendix A5.8. Polymorphic nucleotide sites defining the 55 mitochondrial control region haplotypes for bottlenose dolphins in the North-East Atlantic. Site refers the nucleotide position in the sequences, Hap 1 refers to Haplotype Ttrunc1, 2 to Ttrunc2, etc.

Site / Hap	24	35	84	89	101	140	185	195	196	207	236	239	240	255	256	260	264	265	268	269	270	273	274	284	285	286	294	296	316	320	348	361	382	383	384	444	469	492	493	496	547	593	595	614	630	636
1	T	C	A	C	T	C	T	C	T	A	C	G	C	T	A	C	T	C	T	T	C	C	C	T	C	C	G	T	A	C	T	C	C	A	T	T	C	G	T	A	T	A	G	A	A	G
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47	C	.	.	T	C	T	.	.	.	T	T	.	T	.	C	T	.	.	.	C	.	T	.	.	.	.	A	.	.	.	C	T	T	C	.	.	.	A	.	.	.	.	.	.	.	.
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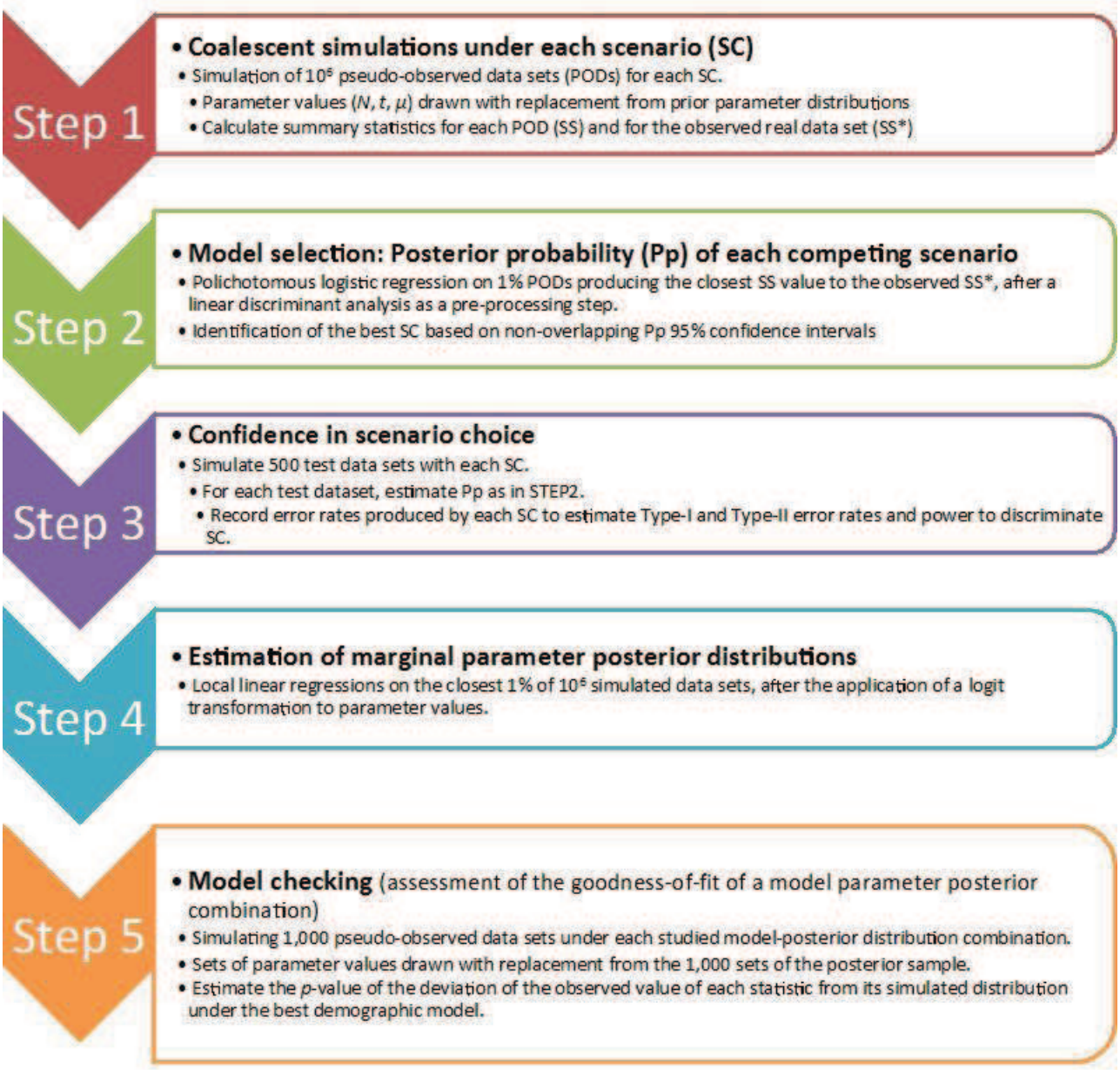
Appendix A5.9. Haplotype frequencies by group and population. GENBANK AN refers to GENBANK Accession Numbers, Coastal S to Coastal South, Coastal N to Coastal North, Pelagic A to Pelagic Atlantic, Pelagic M to Pelagic Mediterranean, Unknown to individuals that were not included in microsatellites analyses (due to amplification issues), and thus were not assigned to any population.

Haplotype	GENBANK AN	Global (N = 369)	Coastal (N=191)	Coastal S (N = 115)	Coastal N (N = 76)	Pelagic (N = 152)	Pelagic A (N = 101)	Pelagic M (N = 51)	Unknown (N = 26)
Ttrunc1	KF650783	0.190	0.335	0.217	0.513	0.000	0.000	0.000	0.231
Ttrunc2	KF650784	0.260	0.466	0.670	0.158	0.013	0.020	0.000	0.192
Ttrunc3	KF650785	0.051	0.094	0.096	0.092	0.000	0.000	0.000	0.038
Ttrunc4	KF650786	0.035	0.010	0.017	0.000	0.072	0.109	0.000	0.000
Ttrunc5	KF650787	0.046	0.084	0.000	0.211	0.000	0.000	0.000	0.038
Ttrunc6	KF650788	0.005	0.000	0.000	0.000	0.013	0.020	0.000	0.000
Ttrunc7	KF650789	0.019	0.000	0.000	0.000	0.039	0.059	0.000	0.038
Ttrunc8	KF650790	0.030	0.000	0.000	0.000	0.066	0.099	0.000	0.038
Ttrunc9	KF650791	0.046	0.000	0.000	0.000	0.105	0.109	0.098	0.038
Ttrunc10	KF650792	0.005	0.000	0.000	0.000	0.013	0.020	0.000	0.000
Ttrunc11	KF650793	0.008	0.000	0.000	0.000	0.020	0.020	0.020	0.000
Ttrunc12	KF650794	0.014	0.000	0.000	0.000	0.033	0.050	0.000	0.000
Ttrunc13	KF650795	0.051	0.000	0.000	0.000	0.125	0.188	0.000	0.000
Ttrunc14	KF650796	0.005	0.000	0.000	0.000	0.007	0.010	0.000	0.038
Ttrunc15	KF650797	0.016	0.000	0.000	0.000	0.026	0.020	0.039	0.077
Ttrunc16	KF650798	0.005	0.000	0.000	0.000	0.007	0.000	0.020	0.038
Ttrunc17	KF650799	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc18	KF650800	0.005	0.000	0.000	0.000	0.013	0.000	0.039	0.000
Ttrunc19	KF650801	0.011	0.000	0.000	0.000	0.026	0.000	0.078	0.000

Ttrunc20	KF650802	0.014	0.000	0.000	0.000	0.033	0.000	0.098	0.000
Ttrunc21	KF650803	0.035	0.000	0.000	0.000	0.086	0.010	0.235	0.000
Ttrunc22	KF650804	0.003	0.000	0.000	0.000	0.007	0.000	0.020	0.000
Ttrunc23	KF650805	0.003	0.000	0.000	0.000	0.007	0.000	0.020	0.000
Ttrunc24	KF650806	0.011	0.000	0.000	0.000	0.020	0.000	0.059	0.038
Ttrunc25	KF650807	0.011	0.000	0.000	0.000	0.026	0.000	0.078	0.000
Ttrunc26	KF650808	0.003	0.000	0.000	0.000	0.007	0.000	0.020	0.000
Ttrunc27	KF650809	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc28	KF650810	0.005	0.000	0.000	0.000	0.013	0.000	0.039	0.000
Ttrunc29	KF650811	0.005	0.010	0.000	0.026	0.000	0.000	0.000	0.000
Ttrunc30	KF650812	0.027	0.000	0.000	0.000	0.046	0.000	0.137	0.115
Ttrunc31	KF650813	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc32	KF650814	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc33	KF650815	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc34	KF650816	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc35	KF650817	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc36	KF650818	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc37	KF650819	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc38	KF650820	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.038
Ttrunc39	KF650821	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc40	KF650822	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.038
Ttrunc41	KF650823	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc42	KF650824	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc43	KF650825	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc44	KF650826	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc45	KF650827	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc46	KF650828	0.008	0.000	0.000	0.000	0.020	0.030	0.000	0.000
Ttrunc47	KF650829	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc48	KF650830	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000

Ttrunc49	KF650831	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc50	KF650832	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc51	KF650833	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc52	KF650834	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc53	KF650835	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc54	KF650836	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Ttrunc55	KF650837	0.003	0.000	0.000	0.000	0.007	0.010	0.000	0.000
	Total	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

3) Appendix Chapter 6



Appendix A6.1. Flow chart about inference of population history using ABC in program DIYABC.

Appendix A6.2. Supplementary details on the ABC analyses

Mutation model

Coalescent simulations assume a mutation model for each type of loci. The mutation model for microsatellite loci was a generalized stepwise-mutation (GSM) model (Estoup *et al.* 2002) with two parameters: a mean mutation rate ($\overline{\mu}_{mic}$) and mean of the geometric distribution for the length, in repeat numbers, of mutation events ($\overline{P}$) drawn from uniform prior distributions ($\overline{\mu}_{mic}$: [10^{-3} – 10^{-4}] and $\overline{P}$: [0.1-0.3], see Appendix A6.3). We accounted for variation in μ_{mic} and P among loci by drawing their individual values from a gamma distribution (Appendix A6.3). These settings allowed for large mutation rate variance across loci (i.e., range of 10^{-5} to 10^{-2}). We also considered mutations inserting or deleting a single nucleotide in the microsatellite sequence.

We used jModelTest 2.1 (Darriba *et al.* 2012) to identify the best substitution model and estimated its parameter. The best mutation model describing the mtDNA sequence evolution was a HKY+I+G model (Hasegawa *et al.* 1985) with a proportion of constant sites of 86.5%, and a shape of the gamma distribution of mutations among sites equal to 0.63 (Appendix A6.3). We assumed a per-site and per-generation mutation rate ranging uniformly between 1×10^{-7} and 1×10^{-5}, as found in the literature (Alter & Palumbi 2009; Fontaine *et al.* 2010).

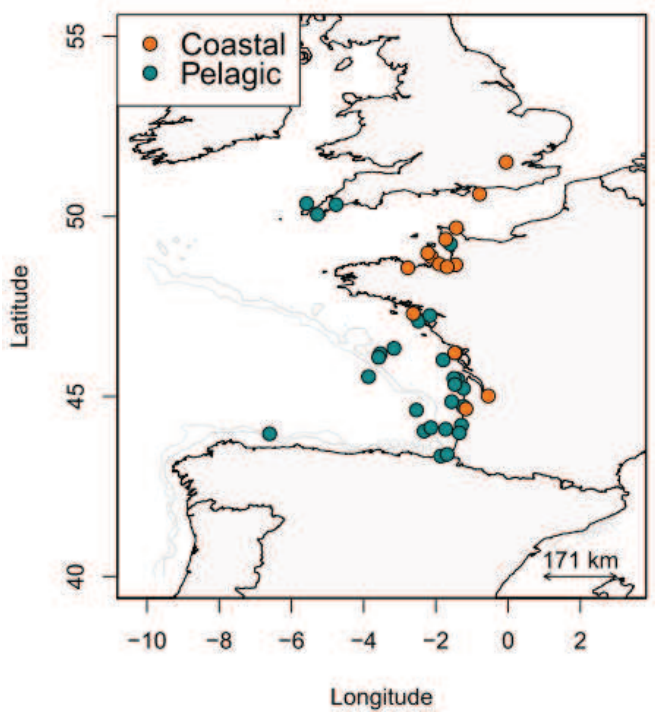
Summary statistics

Overall, 78 summary statistics describing within- and among population genetic diversity were calculated in DIYABC. Within population statistics for microsatellites included the mean number of alleles per locus, expected heterozygosity, allele size variance, M_{GW} statistic of Garza & Williamson across loci (Garza & Williamson 2001). Between population statistics for microsatellites included the mean number of alleles between two populations, F_{ST} (Weir & Cockerham 1984), shared allele distance (Chakraborty & Jin 1993), and $(\delta\mu)^2$ Goldstein's distance (Goldstein *et al.* 1995). For the mtDNA data, the descriptive statistics within populations include the number of segregating sites, the mean pairwise differences and its variance, Tajima's D, and the number of private segregating sites. Statistics computed between groups were the mean of within sample pairwise differences, mean of between sample pairwise differences, and H_{ST} between two samples (Hudson *et al.* 1992).

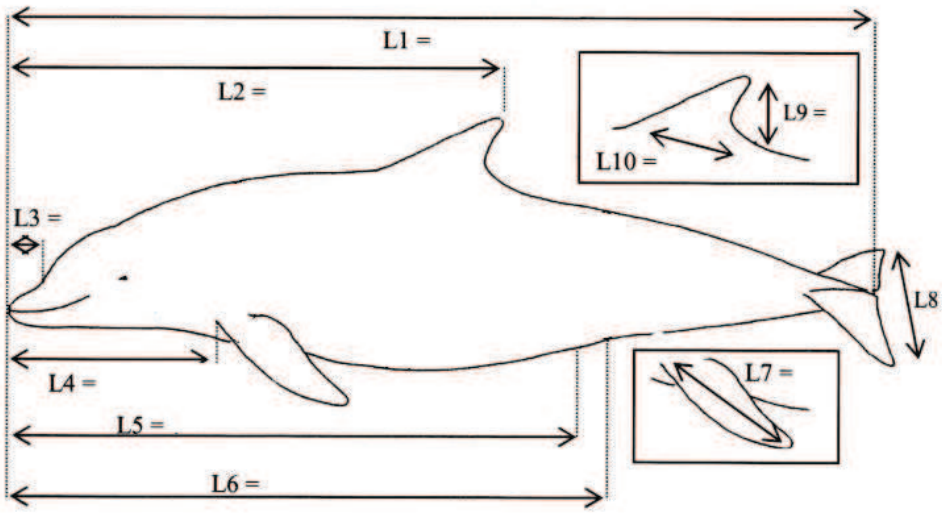
Appendix A6.3. Model specification, prior distributions for demographic parameters and mutation model parameters for the ABC analysis (Figure 6.2).

Demographic Parameter	**Type**	**Prior**
N1	N	UN~[1 – 4,000]
N2	N	UN~[1 – 4,000]
N3	N	UN~[1 – 15,000]
N4	N	UN~[1 – 10,000]
Na	N	UN~[10 – 10,000]
t1 (≤ *t3*)	T	UN~[10 – 5,000]
t2 (≤ *t3*)	T	UN~[10 – 5,000]
t3 (≤ *t4*)	T	UN~[100 – 5,000]
t4	T	UN~[100 – 5,000]
tr	T	UN~[10 – 5,000]
DB	T	UN~[5 – 15]
NBN	N	UN~[1 – 50]
Microsatellites mutation parameter		GSM (40 steps allowed)
$\overline{\mu}_{mic}$		UN~[1 x 10^{-4} – 1 x10^{-3}]
$G_{\mu mic}$		GA~[1 x 10^{-5}, 1 x 10^{-2}, $\overline{\mu}_{mic}$, 2]
$\overline{P}$		UN~[1 x 10^{-1}, 3 x 10^{-1}]
GP		GA~[1 x10^{-2}, 9 x 10^{-1}, $\overline{P}$, 2]
SNI		LU~[1 x10^{-8}, 1 x10^{-5}]
GSNI		GA~[1 x10^{-9}, 1 x10^{-4}, SNI, 2]
MtDNA mutation parameter		HKY (*p-inv*: 86.5, α: 0.634)
μ_{seq}		UN~[1 x 10^{-7}, 1 x 10^{-5}]
K1		UN~[0.050, 20]

Type of parameters: (*N*) effective population size, (*t*) time of the event in generation. Uniform distribution (UN) with 2 parameters: min and max; Gamma distribution (GA) with 4 parameters: min, max, mean and shape; Log-Uniform (LU) distribution with 2 parameters: min and max. See Figure 6.2 for the demographic parameters of each model tested. The mutation model parameters for the microsatellite loci were the mean mutation rate (μ_{mic}), the parameter determining the shape of the gamma distribution of individual loci mutation rate (*P*), and the Single Insertion Nucleotide rate (*SNI*). The Mt-DNA mutation model was a HKY with two variable parameters, the per-site and generation mutation rate (μ_{seq}) and the transition/transversion ratio (*K1*) parameter, and two fixed parameters, the proportion of constant sites (*p-inv.*), and the shape of the Gamma distribution of mutations among sites (α).



Appendix A6.4. Sampling locations and genetic group of origin for individuals included in ecology and/or morphometric analyses ($N_{Coastal}$ = 21 and $N_{Pelagic}$ = 42).



Appendix A6.5. External morphometric measurements of stranded bottlenose dolphins.

Appendix A6.6. Model choice procedure and ABC performance analysis for step *a* in Figure 6.2.

	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9	SC10	SC11
Post. Pr	3.1%	**64.7%**	27.5%	0.0%	0.1%	0.1%	0.0%	1.4%	2.0%	0.1%	1.2%
95%CI	[0.0 – 8.6]	**[62.6 – 66.7]**	[24.0 – 31.0]	[0 – 5.7]	[0 – 5.8]	[0 – 5.8]	[0 – 5.7]	[0 – 7.0]	[0 – 9.0]	[0 – 5.7]	[0 – 6.8]
Confidence in SC selection											
	SC1	**SC2**	**SC3**	**SC4**	**SC5**	**SC6**	**SC7**	**SC8**	**SC9**	**SC10**	**SC11**
D1	60.6%	**9.4%***	5.6%	4.2%	6.8%	8.8%	3.0%	5.8%	6.6%	9.6%	7.6%
D2	**3.2%†**	**68.6%**	**22.4%†**	**0.2%†**	**0.2%†**	**0.2%†**	**0.0%†**	**4.8%†**	**2.2%†**	**1.4%†**	**0.2%†**
D3	1.2%	**16.8%***	66.0%	0.0%	0.2%	0.0%	0.0%	1.2%	3.4%	0.4%	0.2%
D4	2.0%	**0.0%***	0.2%	70.6%	11.0%	0.2%	0.2%	0.2%	6.4%	2.8%	1.6%
D5	1.8%	**0.0%***	0.0%	12.4%	68.0%	0.0%	0.0%	0.2%	1.4%	3.8%	4.6%
D6	4.2%	**0.4%***	0.0%	0.2%	0.4%	62.8%	18.0%	2.4%	1.0%	6.8%	5.0%
D7	0.8%	**0.0%***	0.0%	0.0%	0.0%	9.0%	70.6%	2.4%	0.0%	0.2%	1.2%
D8	1.6%	**2.0%***	1.8%	0.2%	0.0%	0.6%	3.6%	55.8%	14.2%	7.4%	0.2%
D9	3.0%	**1.8%***	2.6%	3.0%	1.0%	0.2%	0.2%	13.2%	51.4%	6.4%	0.2%
D10	12.8%	**0.2%***	1.0%	3.0%	6.0%	8.8%	1.8%	13.0%	12.0%	60.2%	1.6%
D11	8.8%	**0.8%***	0.4%	6.2%	6.4%	9.4%	2.6%	1.0%	1.4%	1.0%	77.6%
Model check (number of outlying statistics)											
$P < 0.05$	12	**8**	7	15	14	13	13	11	11	13	16
$P < 0.01$	2	**1**	2	5	2	3	7	3	1	5	6
$P < 0.001$	2	**1**	1	3	3	2	4	2	2	3	2

D – proportion of case in which the simulation-based model choice procedure was able to select a scenario as the most probable with non-overlapping confidence intervals of the posterior probabilities of each scenario. * Type-I or α-error rate. † Type-II or β-error rate and 1- Σ βi provides the power of the model choice procedure.

Appendix A6.7. Model choice procedure and ABC performance analysis for step *b* in Figure 6.2.

Model selection	**SC1**	**SC2**	**SC3**	**SC4**
Post. prob.	0.7%	15.7%	29.2%	**54.4%**
95CI	[0.0 – 2.5]	[14.2–17.3]	[27.1–31.3]	**[53.0–55.7]**

Confidence in model choice				
	SC1	**SC2**	**SC3**	**SC4**
D1	95.60%	11.00%	13.40%	13.6%*
D2	2.00%	37.40%	17.20%	22.4%*
D3	1.40%	19.20%	44.80%	22.4%*
D4	1.0%†	32.4%†	24.6%†	**41.60%**

Model check (number of outlying statistics)				
$P < 0.05$	15	8	10	7
$P < 0.01$	3	1	0	1
$P < 0.001$	2	1	2	1

D – proportion of case in which the simulation-based model choice procedure was able to select a scenario as the most probable with non-overlapping confidence intervals of the posterior probabilities of each scenario. * Type-I or α-error rate. † Type-II or β-error rate and 1- Σ βi provide the power of the model choice procedure.

Appendix A6.8. Model choice procedure and ABC performance analysis for step *c* in Figure 6.2.

	SC1	SC2	SC3	SC4	SC5
Post. Pr	35.7%	36.7%	17.7%	5.5%	4.4%
95%CI	[35.0 – 36.5]	[36.0 – 37.4]	[17.1 – 18.3]	[5.0 – 6.0]	[3.9 – 4.9]

Confidence in model choice					
	SC1	**SC2**	**SC3**	**SC4**	**SC5**
D1	**51.4%**	36.4%*,††	33.0%††	26.0%††	25.8%††
D2	21.6%†,**	**27.0%**	17.0%†	12.6%†	11.6%†
D3	11.2%**	14.6%*	**30.4%**	8.8%	12.4%
D4	10.8%**	14.4%*	11.0%	**34.4%**	20.0%
D5	5.0%**	7.6%*	8.6%	18.2%	**30.2%**

Model check (number of outlying statistics)					
$P < 0.05$	7	7	2	7	5
$P < 0.01$	1	1	3	1	1
$P < 0.001$	1	1	0	1	1

D – proportion of case in which the simulation-based model choice procedure was able to select a scenario as the most probable with non-overlapping confidence intervals of the posterior probabilities of each scenario. * Type-I or α-error rate for SC2. † Type-II or β-error rate and 1- Σ βi provide the power of the model choice procedure for SC2. ** Type-I or α-error rate for SC1. †† Type-II or β-error rate and 1- Σ βi provide the power of the model choice procedure for SC1.

Appendix A6.9. Model check procedure for the step *c* in Figure 6.2.

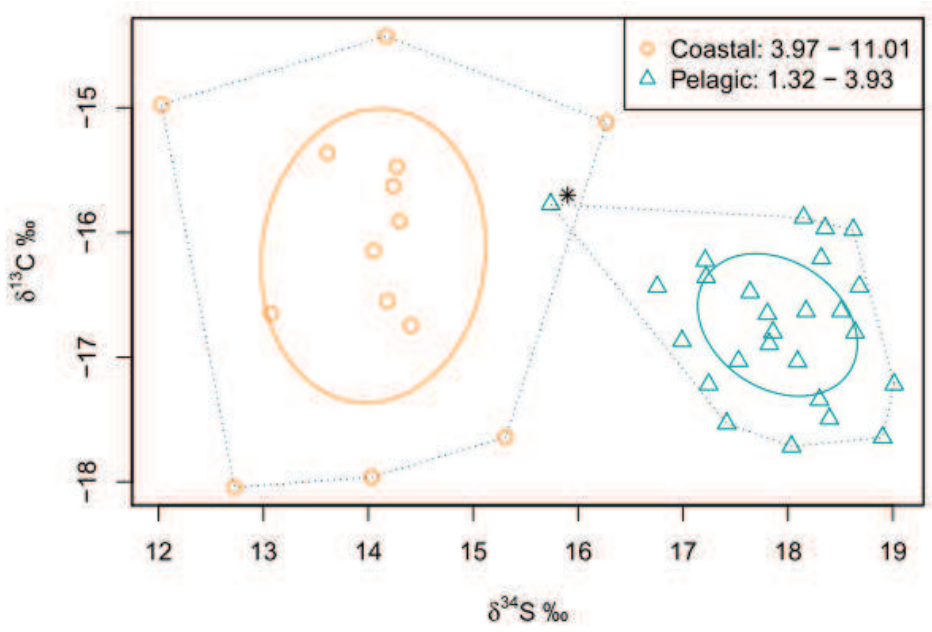
Statistics	Observed	*Prob.* ($S_{simul.} < S_{obs.}$)				
		SC1	SC2	SC3	SC4	SC5
Microsatellites						
MGW_3	0.7616	0.0285 (*)	0.0190 (*)	0.0055 (**)	0.0165 (*)	0.0145 (*)
FST_1&2	0.059	0.917	0.9665 (*)	0.454	0.916	0.6405
MtDNA						
NHA_1	4	0.0030 (**)	0.0070 (**)	0.0055 (**)	0.0015 (**)	0.0035 (**)
NHA_2	5	0.0170 (*)	0.0260 (*)	0.0765	0.0285 (*)	0.052
MPD_1	0.8818	0.0420 (*)	0.0705	0.0635	0.0440 (*)	0.0445 (*)
DTA_1	-1.5873	0.0190 (*)	0.0300 (*)	0.0230 (*)	0.0195 (*)	0.0190 (*)
MNS_1	4.8333	0.0305 (*)	0.0460 (*)	0.0470 (*)	0.0255 (*)	0.0395 (*)
NH2_1&2	6	0.0000 (***)	0.0005 (***)	0.0040 (**)	0.0000 (***)	0.0005 (***)
NH2_1&4	19	0.0385 (*)	0.0420 (*)	0.0605	0.0350 (*)	0.0450 (*)
HST_1&2	0.4054	0.9530 (*)	0.9670 (*)	0.907	0.9525 (*)	0.9315

Evolutionary scenarios SC1 to SC5 are represented in Figure 6.2c. The probability *Prob.*($S_{simul.} < S_{obs.}$) given for each summary statistic was calculated from 1,000 pseudo-observed datasets simulated from the posterior distributions of parameters obtained under the focused scenario. Corresponding tail-area probabilities (*P*-values) were obtained as *Prob.* ($S_{simul.} < S_{obs.}$) and 1.0 - *Prob.* ($S_{simul.} < S_{obs.}$) for *Prob.* ($S_{simul.} < S_{obs.}$) ≤ 0.5 and > 0.5, respectively (*, **, *** = tail-area probability < 0.05, < 0.01 and < 0.001, respectively). In addition to the statistics used during the model choice procedure, the model check procedure used also the two sample statistics including the mean genetic diversity, mean size variance, and the classification index for microsatellite loci. For mtDNA the model check procedure used also within sample statistics including the number of haplotype, mean number of the rarest nucleotide at segregating sites and its variance, and the two sample statistics comprising the number of haplotypes and number of segregating sites. Only significant summary statistics for at least one scenario are shown. Abbreviations for the summary statistics are as follows: Mean Garcia-Williamson index (MGW), FST-statistics (FST); number of mtDNA haplotypes (NHA), mean pairwise differences (MPD); Tajima's D (DTA); mean number of the rarest nucleotide at segregating sites (MNS); the number of distinct haplotypes in two pooled samples (NH2); HST value between populations. Numbers following each statistics refer to the population(s) considered following Figure 6.2.

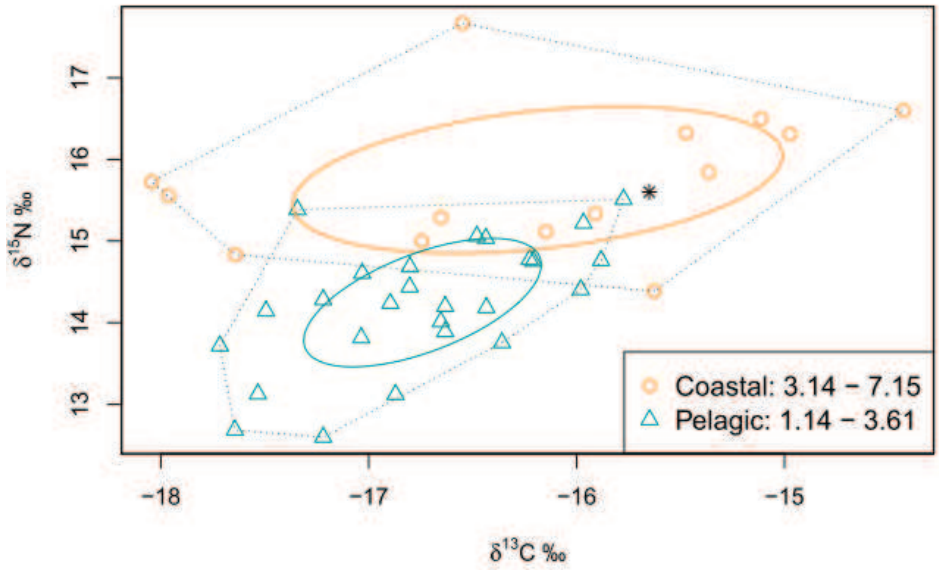
Appendix A6.10. Parameter estimation from scenario SC1 and 2 combined (in Figure 6.2*c*). Mode, median and *x*% Quantile (Q_x) are provided.

Parameter	Mode	$Q_{2.5}$	Median	$Q_{97.5}$
$N_{1\text{-}CS}$	2,160	864	2,060	3,560
$N_{2\text{-}CN}$	1,990	678	1,960	3,660
$N_{3\text{-}PA}$	12,200	6,360	11,600	14,700
$N_{4\text{-}PM}$	4,810	1,500	4,730	9,200
N_a	5,000	766	5,130	9,560
t_1	128	42	133	341
t_2	379	117	447	1,130
t_3	516	215	722	2,390
t_4	2,200	881	2,810	4,860
$\bar{\mu}_{mic}$	2.50E-04	1.58E-04	3.01E-04	6.60E-04
$\bar{P}$	2.68E-01	1.51E-01	2.53E-01	3.00E-01
SNI	1.00E-08	1.00E-08	1.60E-08	1.50E-07
μ_{seq}	2.32E-06	1.43E-06	2.55E-06	5.55E-06
K1	1.12E+01	5.47E-01	1.00E+01	1.94E+01

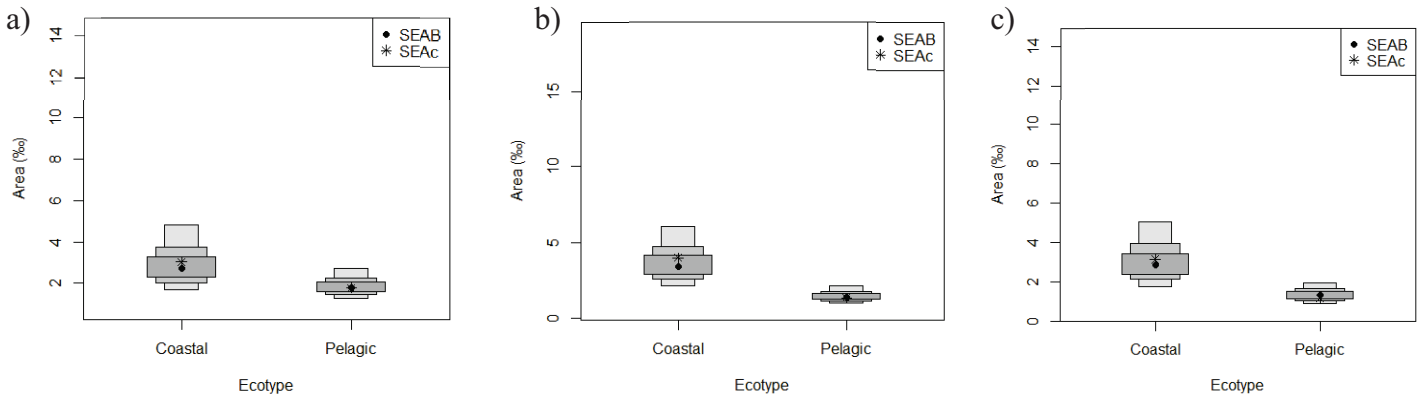
N is expressed in number of diploid individuals, times (*t*) are provided in generation before present.



Appendix A6.11a. $\delta^{34}S$ and $\delta^{13}C$ signatures for coastal and pelagic bottlenose dolphins. Solid lines indicated Standard Ellipses Areas corrected for small sample sizes (SEA_c) and dotted lined Convex Hull Areas. Areas values (‰²) are given in the legend. The star indicates the possible migrant.



Appendix A6.11b. $\delta^{13}C$ and $\delta^{15}N$ signatures for coastal and pelagic bottlenose dolphins. Solid lines indicated Standard Ellipses Areas corrected for small sample sizes (SEA_c) and dotted lined Convex Hull Areas. Areas (‰²) values are given in the legend. The star indicates the possible migrant.



Appendix A6.12a to A6.12c. Measures of uncertainty of Bayesian ellipse areas (SEA_B) based on 10^6 posterior draws indicating 95, 75 and 50% credibility intervals from light to dark grey respectively for a) $\delta^{34}S$ and $\delta^{15}N$, b) $\delta^{13}C$ and $\delta^{34}S$ and c) $\delta^{13}C$ and $\delta^{15}N$. Black dots represent the mode of SEA_B and SEA_c.

4) Appendix of Chapter 7 – General discussion

Appendix 7.A1. Genetic structure analysis within the Coastal South population.

Genetic structure analyses at the scale of the North-East Atlantic placed bottlenose dolphins biopsied in the Normano-Breton gulf in the same cluster as stranded animals in France, in particular in the English Channel but there was also three stranded individuals from a small group of five individuals from the Bassin d'Arcachon (Bay of Biscay, France) that has now disappeared, and stranded dolphins in Galicia (the "Coastal South" population in Chapter 5).

TESS was run only on the individuals of the Coastal South population using the same steps and parameters as given in Chapters 4 and 5 to test for finer-scale population structure. TESS identified two populations corresponding to individuals sampled in France, in majority in the Normano-Breton gulf and individuals stranded in Galicia (Figure A7.1). Genetic differentiation was significant between the two populations identified by TESS (F_{ST} = 0.08, P < 0.001).

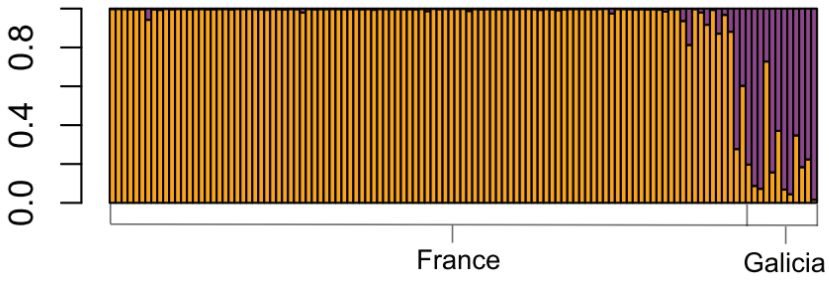


Figure A7.1. TESS membership proportions of bottlenose dolphins sampled in France (in majority in the Normano-Breton gulf) and Galicia. Each vertical column corresponds to one individual, with the colors representing the membership proportions of its genome to each population.

5) Abstracts of each result chapter

Chapter 3: Social structure and abundance of coastal bottlenose dolphins, *Tursiops truncatus*, in the Normano-Breton gulf, English Channel.

A large but poorly studied bottlenose dolphin community, *Tursiops truncatus,* inhabits coastal waters of Normandy (Normano-Breton gulf, English Channel, France). We assessed the social structure and abundance of this community using photo-identification techniques. Like other bottlenose dolphin community worldwide, we found that this resident community has a fission-fusion social structure with fluid associations among individuals (Half-Weight Index = 0.09, SD = 0.136). Association patterns were highly variable as indicated by a high social differentiation (S = 0.95 ± 0.03). The majority of associations were casual, lasting days to months. However, individuals exhibited also a smaller proportion of long-term relationships. Group sizes were large (mean = 25) in comparison with other resident coastal communities, and variable (range: 1 to 100), which could be the results of ecological conditions, in particular resource predictability and availability. Analyses also showed that the community was organized in three social clusters that were not completely isolated from each other. Abundance was estimated at 420 dolphins (95% CI: 331-521), making this coastal community one of the largest identified along European coastlines. Long-term demographic monitoring of these dolphins will be critical for its management, as human activities in the gulf are expected to increase in the upcoming years.

Key words: abundance, bottlenose dolphin, fission-fusion, mark-recapture, photo-identification, social structure.

Chapter 4: Evaluating the influence of ecology, kinship and phylogeography on the social structure of resident coastal bottlenose dolphins

Animal social structures are shaped by external environmental factors and individual intrinsic behavioral traits. They represent a balance between the costs and benefits of group-living to maximize individual fitness. In fission-fusion societies, relationships are highly flexible and influenced by ecological conditions. Bottlenose dolphin societies are fission-fusion, which are variable in terms of association strength, influence of kinship and relationships between or within sexes throughout the wide range of habitats where they are found. Here, a combination of markers and analyses were used to study the population structure and the drivers of social structure in coastal bottlenose dolphins of the Normano-Breton gulf (English Channel). While a single population was identified using genetics, stable isotopes revealed three ecological clusters, consistent with previous social structure analyses based on photo-identification data. In contrast to most studied bottlenose dolphin populations, and many fission-fusion species, individuals did not preferentially associate with kin. Instead they associate with individuals of similar ecology. Bottlenose dolphins in coastal waters of the North-East Atlantic may have been more recently founded from a pelagic population than in other parts of the world. This suggests that coastal bottlenose dolphin social structure might have been derived from a pelagic social organization. Thus, a combination of ecological conditions, in particular resource availability and the absence of predators, individual behavioral preferences and population structure history may shape dolphin social organization. We emphasize that stable isotope analysis is a promising tool to investigate the link between social structure and foraging ecology, particularly in difficult to observe taxa.

Keywords: social structure, ecological specializations, relatedness, population genetics, stable isotopes.

Chapter 5: Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic

Despite no obvious barrier to gene flow, historical environmental processes and ecological specializations can lead to genetic differentiation in highly mobile animals. Ecotypes emerged in several large mammal species as a result of niche specializations and/or social organization. In the North-West Atlantic, two distinct bottlenose dolphin (*Tursiops truncatus*) ecotypes (i.e. "coastal" and "pelagic") have been identified. Here, we investigated the genetic population structure of North-East Atlantic (NEA) bottlenose dolphins on a large scale through the analysis of 381 biopsy-sampled or stranded animals using 25 microsatellites and a 682 bp portion of the mitochondrial control region. We shed light on the likely origin of stranded animals using a carcass drift prediction model. We showed, for the first time, that coastal and pelagic bottlenose dolphins were highly differentiated in the NEA. Finer-scale population structure was found within the two groups. We suggest that distinct founding events followed by parallel adaptation may have occurred independently from a large Atlantic pelagic population in the two sides of the basin. Divergence could be maintained by philopatry possibly as a result of foraging specializations and social organization. As coastal environments are under increasing anthropogenic pressures, small and isolated populations might be at risk and require appropriate conservation policies to preserve their habitats. While genetics can be a powerful first step to delineate ecotypes in protected and difficult to access taxa, ecotype distinction should be further documented through diet studies and the examination of cranial skull features associated with feeding.

Keywords: population genetics, ecotypes, philopatry, feeding specializations, conservation, cetaceans

Chapter 6: Ecological opportunities and specializations shaped genetic divergence in a highly mobile marine top predator

Environmental conditions can shape genetic and morphological divergences. Releases of new habitats during past environmental changes were a major driver of evolutionary diversification. Here, the forces shaping population structure and ecotype differentiation ("pelagic" and "coastal") of bottlenose dolphins in the North-East Atlantic were investigated using complementary evolutionary and ecological approaches. Using Approximate Bayesian Computation population history analyses, we showed that coastal populations were founded by the Atlantic pelagic population after the Last Glacial Maxima probably as a result of newly available coastal ecological niches. Pelagic dolphins from the Atlantic and the Mediterranean Sea diverged during a period of high productivity in the Mediterranean Sea. Genetic differentiation between the coastal and pelagic ecotypes is likely maintained by niche specializations, as indicated by stable isotope and stomach content analyses, and social behavior. The two ecotypes were only weakly morphologically segregated in contrast to other parts of the world. This may be linked to weak contrasts between coastal and pelagic habitats and/or a relatively recent divergence. We suggest that ecological opportunity to specialize is a major driver of genetic and morphological divergences. Combining genetic, ecological and morphological approaches is essential to understand population structure of mobile and cryptic species.

Key words: ecological niches, demographic history, genetic structure, morphology, bottlenose dolphins

6) References cited in the Appendix

Alter SE, Palumbi SR (2009) Comparing evolutionary patterns and variability in the mitochondrial control region and cytochrome b in three species of baleen whales. *Journal of Molecular Evolution* **68**, 97-111.

Chakraborty R, Jin L (1993) A unified approach to study hypervariable polymorphisms: statistical considerations of determining relatedness and population distances. *EXS* **67**, 153-175.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772-772.

Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology* **11**, 1591-1604.

Fontaine MC, Tolley KA, Michaux JR, Birkun A, Ferreira M, Jauniaux T, Llavona A, Ozturk B, Ozturk AA, Ridoux V, Rogan E, Sequeira M, Bouquegneau JM, Baird SJE (2010) Genetic and historic evidence for climate-driven population fragmentation in a top cetacean predator: the harbour porpoises in European water. *Proceedings of the Royal Society B-Biological Sciences* **277**, 2829-2837.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology* **10**, 305-318.

Goldstein DB, Linares AR, Cavallisforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463-471.

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate *F*-statistics. *Journal of Heredity* **86**, 485-486.

Hasegawa M, Kishino H, Yano TA (1985) Dating of the human ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**, 160-174.

Hoelzel AR, Potter CW, Best PB (1998) Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 1177-1183.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics* **132**, 583-589.

Kingston SE, Adams LD, Rosel PE (2009) Testing mitochondrial sequences and anonymous nuclear markers for phylogeny reconstruction in a rapidly radiating group: molecular systematics of the Delphininae (Cetacea: Odontoceti: Delphinidae). *Bmc Evolutionary Biology* **9**, 245.

Krützen M, Valsecchi E, Connor RC, Sherwin WB (2001) Characterization of microsatellite loci in *Tursiops aduncus*. *Molecular Ecology Notes* **1**, 170-172.

Litz JA, Hugues CR, Garrison LP, Fieber LA, Rosel PE (2012) Genetic structure of common bottlenose dolphins (*Tursiops truncatus*) inhabiting adjacent South Florida estuaries – Biscayne Bay and Florida Bay. *Journal of Cetacean Research & Management* **12**, 107-117.

Malausa T, Gilles A, Meglécz E, Blanquart H, Duthoy S, Costedoat C, Dubut V, Pech N, Castagnone-Sereno P, Delye C, Feau N, Frey P, Gauthier P, Guillemaud T, Hazard L, Le Corre V, Lung-Escarmant B, Male PJG, Ferreira S, Martin JF (2011) High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources* **11**, 638-644.

Meglécz E, Costedoat C, Dubut V, Gilles A, Malausa T, Pech N, Martin JF (2010) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics* **26**, 403-404.

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* **142**, 1061-1064.

Nater A, Kopps AM, Krützen M (2009) New polymorphic tetranucleotide microsatellites improve scoring accuracy in the bottlenose dolphin *Tursiops aduncus*. *Molecular Ecology Resources* **9**, 531-534.

Quérouil S, Silva MA, Freitas L, Prieto R, Magalhaes S, Dinis A, Alves F, Matos JA, Mendonca D, Hammond PS, Santos RS (2007) High gene flow in oceanic bottlenose dolphins (*Tursiops truncatus*) of the North Atlantic. *Conservation Genetics* **8**, 1405-1419.

Rambaut A, Drummond AJ (2007) Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer.

Rannala B (2013) BayesAss Edition 3.0 User's Manual, University of California Davis.

Raymond M, Rousset F (1995) GENEPOP (version 1.2) - Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Rooney AP, Merritt DB, Derr JN (1999) Microsatellite diversity in captive bottlenose dolphins (*Tursiops truncatus*). *Journal of Heredity* **90**, 228-231.

Rosel PE, Forgetta V, Dewar K (2005) Isolation and characterization of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). *Molecular Ecology Notes* **5**, 830-833.

Rosel PE, Hansen L, Hohn AA (2009) Restricted dispersal in a continuously distributed marine species: common bottlenose dolphins *Tursiops truncatus* in coastal waters of the western North Atlantic. *Molecular Ecology* **18**, 5030-5045.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.

Sellas AB, Wells RS, Rosel PE (2005) Mitochondrial and nuclear DNA analyses reveal fine scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico. *Conservation Genetics* **6**, 715-728.

Valsecchi E, Amos W (1996) Microsatellite markers for the study of cetacean populations. *Molecular Ecology* **5**, 151-156.

Vollmer N (2011) *Population structure of common bottlenose dolphins in coastal and offshore waters of the Gulf of Mexico revealed by genetic and environmental analyses.* PhD dissertation, University of Louisiana.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

7) International conferences

Oral presentations

Louis M., Viricel A., Lucas T., Peltier H., Alfonsi E., Berrow S., Brownlow A., Covelo P., Dabin W., Deaville R., de Stephanis R., Gally F., Gauffier P., Penrose R., Silva M. A., Guinet C. and Simon-Bouhet B. 2013. Population genetics of bottlenose dolphins in the North-East Atlantic and implications for conservation. Society for Marine Mammalogy conference, Dunedin, New Zealand, December 2013.

Louis M. Population structure and evolutionary ecology of bottlenose dolphins in the North-East Atlantic. Oral presentation. Marine Mammal Genomics workshop. Society for Marine Mammalogy conference, Dunedin, New Zealand, December 2013. *American Genetic Association student travel award.*

Louis M., Guinet C., Lucas T., Viricel A., Peltier H., Alfonsi E., Berrow S., Brownlow A., Covelo P., Dabin W., Deaville R., Gally F., Gauffier P., Penrose R., Silva M. A. and Simon-Bouhet B. 2013. Population genetics of bottlenose dolphins in the North-East Atlantic: a pelagic versus coastal segregation. 27th Annual Conference of the European Cetacean Society, Setubal, Portugal, April 2013. *Best student presentation award.*

Louis M., Gally F., Barbraud C., Béesau J., Tixier P., Simon-Bouhet B., Le Rest K. and Guinet C. 2013. Social structure, abundance and conservation of a population of bottlenose dolphins, *Tursiops truncatus*, in the English Channel. Workshop "Bottlenose dolphin's conservation: what we can learn from different resident populations?" Oral presentation. 27th Annual Conference of the European Cetacean Society, Setubal, Portugal, April 2013.

Poster presentation

Louis M., Béesau J., Gally F., Barbraud C. and Guinet C. Demography and social structure of a bottlenose dolphin population in the English Channel. Poster, 25th Annual Conference of the European Cetacean Society, Cadiz, Spain, March 2011.

Presentations I have co-authored

Giménez J., Baron E., **Louis M**., Verborgh P., Gauffier P., Forero M. G., Eljarrat E., Barcelo D. and de Stephanis R. 2013. A multi-disciplinary approach to define conservation units of bottlenose dolphins (*Tursiops truncatus*). Oral presentation. 28th Annual Conference of the European Cetacean Society, Liège, Belgium. *presented by Joan Giménez*.

Giménez J., Baron E., **Louis M**., Verborgh P., Gauffier P., Forero M. G., Eljarrat E., Barcelo D. and de Stephanis R. 2013. A multi-disciplinary approach to define conservation units of bottlenose dolphins (*Tursiops truncatus*). Poster. Society for Marine Mammalogy conference, Dunedin, New Zealand, December 2013. *presented by Joan Giménez*.

Structures sociale, écologique et génétique du grand dauphin, *Tursiops truncatus*, dans le golfe Normand-Breton et dans l'Atlantique Nord-Est

Résumé :
Les patrons de structuration des espèces animales à fine et à large échelles peuvent être façonnés par des facteurs environnementaux et des traits comportementaux individuels. Les objectifs de cette thèse combinant des approches sociales, génétiques, isotopiques et morphométriques sont de décrire et comprendre i) les structures sociale, écologique et génétique de la population de grands dauphins du golfe Normand-Breton (NB) et ii) la structure de population de l'espèce à l'échelle de l'Atlantique Nord-Est (ANE). Les grands dauphins du golfe NB forment une unique population génétique qui est composée de trois ensembles sociaux et écologiques distincts. Les associations entre individus semblent être influencées par l'écologie et non par les liens de parenté. La structure génétique du grand dauphin à l'échelle de l'ANE est hiérarchique, avec deux écotypes, l'un côtier et l'autre pélagique, qui sont chacun divisé en deux populations. Les populations côtières sont issues d'une population pélagique et auraient colonisé les habitats côtiers libérés lors de la dernière déglaciation, ce qui a permis la diversification de l'espèce. Cette structure semble maintenue par les spécialisations écologiques et le comportement social des individus. Par ailleurs, l'origine pélagique des grands dauphins du golfe NB pourrait expliquer certains de leurs traits sociaux. Pour conclure, les patrons de structuration à fine et à large échelles de ce prédateur supérieur semblent influencés par les comportements sociaux et écologiques, les conditions environnementales présentes et passées ainsi que par son histoire évolutive. L'absence de différences morphologiques marquées entre les deux écotypes pourrait s'expliquer par leur divergence relativement récente ou par un faible contraste entre les habitats pélagiques et côtiers dans l'ANE. Ce travail souligne l'intérêt de combiner de multiples approches à différentes échelles temporelles et spatiales pour comprendre la structure sociale et la structure de population d'espèces mobiles et cryptiques. Ces résultats ont également des implications majeures pour la conservation, en particulier pour la définition d'unités de gestion.

Mots clés : génétique des populations, écologie, structure sociale, histoire démographique, grands dauphins

Social, ecological and genetic structures of bottlenose dolphins, *Tursiops truncatus,* in the Normano-Breton gulf and in the North-East Atlantic

Abstract:
Complex interactions between environmental factors and behavioral traits may shape the fine and large scale structuring patterns of animal species. The objectives of this dissertation were to describe and understand i) the fine-scale social, ecological and genetic structures of bottlenose dolphins in the Normano-Breton (NB) gulf and ii) the population structure of the species at the scale of the North-East Atlantic (NEA) by combining social, genetic, stable isotope and morphometric approaches. Coastal bottlenose dolphins in the NB gulf form a single genetic population subdivided in three social and ecological clusters. Ecology but not kinship may influence association patterns. In the NEA, bottlenose dolphin genetic structure is hierarchical. They form two ecotypes, i.e. coastal and pelagic, each of them being further divided in two populations. This genetic structure was likely triggered by past changes in the environment (i.e. deglaciation) that created ecological opportunities for diversification. Ecological specializations and social behavior may maintain genetic divergence. In turn, the pelagic origin of bottlenose dolphins in the NB gulf may explain some of their social structure traits. Thus, an interaction between social and ecological behaviors, current and past environmental conditions, and evolutionary history may drive the fine and large scale structuring patterns of this top predator. The absence of strong differences in morphology between the two ecotypes may be explained by their relatively recent divergence or by low contrasts between the pelagic and coastal habitats in the NEA. This work highlights the power of combining approaches at different temporal and spatial scales for assessing the social and population structures of highly mobile and difficult to access species. The results have also major conservation implications especially for the designation of management units.

Keywords: population genetics, ecology, social structure, demographic history, bottlenose dolphins

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